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An Ecological Approach to the Taxonomy of the Genus Alloglossidium (Trematoda: Macroderoididae).

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AN ECOLOGICAL APPROACH TO THE TAXONOMY OF THE GENUS *ALLOGLOSSIDIUM* (TREMATODA:MACRODEROIDIDAE)

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ABSTRACT

All species of Alloglossidium except A. corti attain sexual maturity in invertebrates. A. corti uses a wide range of arthropods as second intermediate hosts and becomes sexually mature only in catfishes. A second species of Alloglossidium closely resembles A. corti with regard to host preference, but attains sexual maturity while encysted within the antennary gland of the crayfish Procambarus (Pennides) spiculifer. A third species of Alloglossidium uses the fresh-water shrimp as a definitive host and is incapable of parasitizing vertebrates. A. hirudicola has been reported from the leeches Haemopis sp. and Macrobdella decora in Wisconsin. A. macrobdellensis parasitizes the leech M. ditetra in Louisiana.

The host preferences of one species of Alloglossidium are generally exclusive of the host preferences of the other species. In the few instances in which one species of Alloglossidium parasitizes the host of another species, no host induced structural variation occurred.

The presence or absence of any species of Alloglossidium in an aquatic habitat is independent of the presence or absence of any of its congeners. Each species of Alloglossidium is structurally distinct, generally allohospitalic, and ecologically independent of other members of the genus, and therefore, distinct biological species.

A species concept pertaining to digenetic trematodes and the phenomenon of progenesis are discussed. The phylogenetic implications of progenesis are related to digenetic trematodes in general and specifically to the genus Alloglossidium. A phylogenetic scheme for the species of

Alloglossidium based upon adult structure, host specificity, and degree of progenesis is proposed.

INTRODUCTION

Purpose of Study

The intention of this study is to utilize life history information to determine the biological species of Alloglossidium Simer, 1929. This research was initiated with the discovery of gravid trematodes in the antennary gland of the freshwater shrimp Palaemonetes kadiakensis. These forms were determined to be members of the genus Alloglossidium but could not be assigned to any of the described species.

Life history studies on the genus were conducted to determine if the trematodes from the freshwater shrimp represented an undescribed species and to evaluate the validity of other species assigned to the genus Alloglossidium. Because digenetic trematodes are hermaphroditic, and potentially self-fertilizing organisms, a biological species concept involving reproductive isolation has no practical applications for the taxonomist. Recent studies of intraspecific morphological variability (Watertor, 1967, 1968) have shown that species concepts based upon structural criteria alone are subject to error.

For these reasons I felt that a natural history study of the host-parasite associations within the genus was necessary for a proper determination of the various species of Alloglossidium.

Taxonomic History of Alloglossidium and Macroderoididae

Lamont (1921) described Plagiorchis corti, family Plagiorchiidae, from the tadpole madtom Schilbeodes gyrinus in Wisconsin. Previously, the genus Plagiorchis had not been reported from fishes, but Lamont noted the similarity of P. corti with P. notabilis from the rock pipet, Anthus obscurus. P. ameiurensis (McCoy, 1928) was described from the

yellow bullhead, Ameiurus natalis, based upon size and structural differences from P. corti. Mueller (1930) attributed the differences between P. corti and P. ameiurensis to the brief and inadequate description by Lamont, and placed P. ameiurensis in synonymy. In the same paper, Mueller described P. geminus on the basis of the more limited extent of the vitellaria while conceding that "it is in general unsafe to establish a new species upon a single character, in this case at least there seems no other way of dealing with the facts." Simer (1929), apparently unaware of the work of Lamont and McCoy, erected the genus Alloglossidium for an intestinal parasite of the channel catfish, Ictalurus puncatus, and designated A. kenti as the type species. Simer presented a morphological comparison of his new genus with Glossidium and Haplometroides, but in his short description of A. kenti, he made no comparison with other species.

Van Cleave and Mueller (1934) pointed out that "the species which Simer (1929) described as A. kenti is very clearly a renamed P. corti." They removed P. corti and P. geminus from the genus Plagiorchis as these two species were the only members possessing an I-shaped bladder rather than the more typical Y-shape. The synonymy of A. kenti, they felt, left the genus Alloglossidium available for these two species. At the same time, they allied the two species A. corti and A. geminus with the Allocreadidae. The question of whether Van Cleave and Mueller's assumption that the genus Alloglossidium was available has been raised by Miller in his Ph.D. dissertation (1957). However, the validity of the genus Alloglossidium has not been challenged in print.

Life history studies by McMullen (1935) revealed the relationship

of Alloglossidium corti and the closely related Macroderoides typicus with the family Plagiorchiidae. He stated that the principal difficulty of allying these two genera with the plagiorchiids was the I-shaped bladder of Alloglossidium and Macroderoides. He demonstrated, however, that within the large family Plagiorchiidae, there was a gradation of bladder structure from the Y-type to the I-type and accordingly returned Alloglossidium and Macroderoides to the Plagiorchiidae.

In his discussion of the taxonomy of the Plagiorchiidae, McMullen (1937) reported that on the basis of larval characters, Alloglossidium and Macroderoides belonged to the superfamily Plagiorchioidea but pointed out that

> "these closely related genera show some rather striking differences when compared with other genera of the superfamily. For this reason it is proposed that a new family, the Macroderoididae be established for them. The cercariae of this family are characterized by the I-shaped excretory bladder and the small number of stylet glands. The adults are elongate and spined, the intestinal ceca are long, the oral sucker and acetabulum are about the same size, the genital pore is just anterior to the acetabulum and the vitellaria are heavily massed follicles. As far as known, they are parasitic in the digestive tract of fishes."

Following McMullen's study, the taxonomy of Alloglossidium and Macroderoididae remained stable until the publication of Systema Helminthum by Yamaguti in 1953. Yamaguti disregarded the synonomy of A. kenti with A. corti by Van Cleave and Mueller (1934) and designated A. kenti as the type species of Alloglossidium. This recommendation was not ex-

plained, nor did Yamaguti justify his removal of A. geminus to Glossidium in the same publication. Yamaguti returned Alloglossidium to the Plagiorchiidae based upon McMullen's work in 1935. This error on the part of Yamaguti was probably due to an incomplete search of the literature. This is borne out in Yamaguti's revision (1971) in which Alloglossidium was placed in the family Macroderoididae as established by McMullen (1937). However, the position of A. kenti and G. geminus was not altered.

Alloglossidium hirudicola Schmidt and Chaloupka, 1969 was described as reaching sexual maturity in the leech Haemopis sp. from an unknown locality. Taft and Kordiyak (1973) reported the occurrence of A. hirudicola in Haemopis sp. and Macrobdella decora in Wisconsin and noted morphological differences in the specimens obtained from M. decora. A study was made by Beckerdite (1973) on a sexually mature trematode in M. ditetra in Louisiana and A. macrobdellensis was described by Beckerdite and Corkum in 1974.

Macroderoides progeneticus Sullivan and Heard, 1969 was described from gravid specimens encysted in the antennary gland of the crayfish Procambarus (Pennides) spiculifer. As a result of new evidence derived from my dissertation research, I have proposed a new combination, Alloglossidium progeneticum, on the basis of information regarding the structure and life history of this species (Font and Corkum, 1975). In the same publication, Alloglossidium renale was described from the fresh-water shrimp, Palaemonetes kadiakensis, based upon evidence gained through these life history studies.

In recent years, additional genera have been added to Macroderoididae; Glypthelmins Schell (1962), Choledocystus, Renoldstrema, and Repandum Odening (1964), Paramacroderoides, Haplometrana, and Alloglyptus,

Schell (1970). The findings of Martin (1969) and Sullivan and Byrd (1970) necessitated a revision of the family diagnosis by the latter authors that incorporated characters possessed by the new genera.

In summary, Alloglossidium is presently placed in the family Macroderoididae. The genus is comprised of the following species, A. corti, A. hirudicola, A. macrobdellensis, A. progeneticum, and A. renale. The taxonomic position of A. kenti and G. geminus is uncertain.

Life History and Distribution of Alloglossidium

Surveys of the parasites of freshwater fishes include many records of Alloglossidium corti as a common intestinal parasite of catfishes, bullheads, and madtoms (Ictaluridae) in the United States and Canada.

The life cycle of A. corti was first reported by McCoy (1928). An examination of small crayfish revealed the presence of encysted metacercariae which were identified as those of a xiphidiocercaria found commonly in Helisoma trivolvis. A positive identification was made possible by a comparison of the cercarial stylet with the stylet which remained intact within the cyst. Three weeks after feeding infected crayfish to hatchery-reared catfish, McCoy recovered mature trematodes in the intestine which were identified as Plagiorchis ameiurensis (= A. corti). The cysts in the crayfish were located in the muscles at the base of the thoracic appendages, in the muscles between the thorax and abdomen and even in the basal parts of the antennae. Naturally infected crayfish contained seven or fewer cysts, but McCoy was able to infect experimentally crayfish with as many as 100 to 150 cysts. He was also able to infect experimentally dragonfly larvae, but no naturally infected larvae were found in his study. Metacercariae from crayfish and dragonfly larvae were both capable

of infecting the definitive host. McCoy reported sexually mature A. corti in catfish 18 days following exposure to metacercariae in aquaria at 18°C, but estimated that about 35 days were required for the production of the enormous numbers of ova typically found in the uterus. He stated that development was much more rapid in fish kept at 27°C. McCoy was unsuccessful in his attempt to infect the 1st intermediate host (Helisoma trivolvis) with eggs of A. corti.

McMullen (1935) investigated the life histories of A. corti and Macroderoides typicus. The cercariae of A. corti were found to penetrate and encyst in dragonfly and mayfly nymphs. Natural infections of A. corti metacercariae were found in crayfish, dragonfly and mayfly nymphs in the study area. Additional information added to the life history of A. corti was that it was distinct from M. typicus in the morphology of its cercariae, metacercariae, and adults as well as in preference of 2nd intermediate and definitive hosts.

Crawford (1937) studied the relationship of the snail Helisoma trivolvis, the cercariae and metacercariae of A. corti and the dragonfly naiads which served as hosts for the infection. He added to the description of the cercaria of A. corti by noting that the rudiments of the testes were plainly visible in well stained specimens. The cercariae demonstrated a marked nocturnal periodicity, emerging from H. trivolvis between 7:00 and 9:00 P.M. They alternately crawled along the bottom and swam vertically for short periods until drawn into the rectum of a dragonfly nymph by respiratory currents. Crawford identified several susceptible species of dragonflies. After penetration of the rectum, the cercariae encysted among the abdominal muscle fibers within an hour. Within three days a host cyst of spindle shaped cells formed around the

metacercaria. The trematode grew appreciably within the cyst and some genital development occurred. Crawford listed several insusceptible dragonfly hosts in which either penetration did not occur or the cysts became imbedded with a hard brown pigment and the metacercariae died.

Crawford found that the incidence of infection in Helisoma trivolvis was very low. The cercariae did not emerge in the summer, but mature infections developed in the fall and persisted throughout the winter and spring. He reported that A. corti took 17 to 21 days to reach maturity in the bullhead. Similar to previous workers, he was unable to infect Helisoma trivolvis with eggs of A. corti.

In contrast with Crawford's data on emergence of A. corti cercariae, Cort, McMullen and Brackett (1939) found that the summer incidence of A. Corti in Helisoma campanulatum reached as high as 71% of the large snails examined. They also reported a high summer mortality of these snails, due either to the completion of the life span or to the extremely heavy trematode infections.

Sullivan and Heard (1969) reported a small amount of life history data in their description of Macroderoides progeneticus (= A. progeneticum) which they found encysted in the antennary gland of Procambarus (P.) spiculifer. They found that while the incidence of infection in P. (P.) spiculifer was 100%, one other species of crayfish, Cambarus latimanus, which was fairly common in the type locality did not harbor the infection. Various species of fishes from the type locality were fed infected antennary glands but in all cases results were negative. Rana catesbeiana from the type locality were not infected with A. progeneticum but analyses of the stomach contents showed that many P. (P.) spiculifer were consumed by the bullfrogs. The authors believed that "a crayfish-eating vertebrate may serve as a means of egg dispersal rather than a true definitive

host." Eggs of A. progeneticum were fed to lab-reared Physa and Helisoma, but although live miracidia were observed in the snails' feces, no snail infections resulted from this experiment.

Taft and Kordiyak's (1973) study of the incidence, distribution and morphology of A. hirudicola provided the first known locality for this trematode. Eighteen percent of Haemopis sp. in Wisconsin were infected with A. hirudicola. Macrobdella decora, a new host record, had a 30% infection rate with an average of 2.1 worms per parasitized leech. The largest number of helminths recovered from a single leech was 24. The authors believed that the percentages of infected leeches were probably correlated with the relative abundance of snails. Fifty-seven M. decora taken from a farm pond where only Sphaerium sp. was present were uninfected. Highest infection rates were obtained from bodies of water with large populations of Helisoma, Physa and Ferrisia. Corkum and Beckerdite (1975) described A. macrobdellensis and elucidated some aspects of its life history. Two hosts were involved in the life cycle. Cercariae produced in Helisoma trivolvis penetrated the leech, Macrobdella ditetra. The cercariae lost their tails and underwent a period of development in the coelom before encysting in the crop wall. After an additional period of development, the trematodes excysted, moved to the leech's intestine and attained sexual maturity. The annual life cycle of the leech in Louisiana was largely responsible for the seasonality of the trematode infection. Newly hatched M. ditetra appeared shortly after the death of the parent generation in July. Light infections with coelomic forms of A. macrobdellensis occurred within the next month. The numbers of coelomic, crop and intestinal forms gradually increased throughout the winter until a sharp rise in the numbers of all three forms was noted in March. Helisoma

trivolvis infected with cercariae of A. macrobdellensis were found in the study area, but experimental feeding of eggs to lab-reared H. trivolvis did not produce cercarial infections.

In summary, the life cycles and additional life history data are known for A. corti and A. macrobdellensis. Some information has been reported concerning the life histories of A. hirudicola and A. progeneticum.

MATERIALS AND METHODS

Field Collections

Collections of potential hosts of Alloglossidium and Macroderoides were made from various aquatic habitats in four states. Louisiana --- University Lake, Baton Rouge; Mississippi River borrow pits, 6 mi S of Baton Rouge; Whisky Bay pond, 5 mi W of Brusly; Choctaw Bayou, 4 mi W of Brusly; Blind Choctaw Bayou, 8 mi W of Brusly; Mississippi River borrow pit, Brusly; Mississippi River borrow pit, Addis; Lake Verret, Pierre Pass; Mississippi River borrow pit, St. James; Bayou Chevreuil, 7 mi S of Vacherie on Rt. 20; pond, 2 mi E of Head of Island on Rt. 22; roadside canal and swamp, 4 mi S of Sorrento on Rt. 61; Bayou Des Allemands, 3 mi N to 5 mi S of Des Allemands; Bayou Gauche, 2 mi W of Bayou Gauche (town); roadside canals, Rt. 90 between Des Allemands and Bouttee; canals and marsh, 3 mi SW of Paradis; Grand Bayou, 3 mi SE of Paradis; Mississippi River borrow pit, 2 mi NW of Luling; Thompson Creek and ponds, 2 mi SE of Starhill on Rt. 61; Bayou Sara, 4 mi NW of Bains; Lake Rosemound, 2 mi W of Laurel Hill; Alexander Creek, St. Francisville; Alligator Bayou, 3 mi S of St. Francisville; Karr Creek, 3 mi S of Jackson on Rt. 68; Talisheek Creek, Talisheek; Lee's Creek, 4 mi S of Boga-

lusa on Rt. 21; Caddo Lake, 5 mi W of Shreveport; roadside canal, 3 mi N of Holly Beach on Rt. 27; Lake Chicot, 9 mi N of Ville Platte.

Georgia --- Middle Oconee River, 3 mi S of Athens on Rt. 129; Call's Creek, 3 mi E of Watkinsville; Old Oconee River, Whitehall; East Athens Creek, Athens; McKinney's Pond and Stream, 3 mi SE of Midville; pond near Oconee River, 10 mi W of Soperton on Rt. 46; Apalachee River, 15 mi E of Athens on Rt. 78; Mason Mill Creek, 3 mi N of Danielsville on Rt. 29; Little Nails Creek, 1 mi S of Hollingsworth on Rt. 441; sandy creek, 5 mi E of Buffington; Middle Oconee River, 1 mi S of Pendergrass on I-85; Candler Creek, junction of Rt. 323 and Rt. 52; North Oconee River, 3 mi SE of Gillsville on Rt. 323.

Florida --- Sandy Creek, 5 mi E of Panama City on Rt. 22; Chipola River, 1 mi E of Clarksville; Sink Creek, 5 mi S of Marianna on Rt. 73; Sandy Creek, 10 mi E of Defuniak Springs on Rt. 90; sandy creek near Yellow River, on I-10.

Alabama --- Fish River at I-10 near Loxley; Big Creek Lake at Rt. 70; Uphapee Creek, 3 mi N of Tuskegee near I-85.

The most widely used method of collecting in ponds was random dipping with nets among the emergent vegetation. This method was most successful for many species of snails, crayfishes, and fresh-water shrimp, insect larvae, small fishes, and tadpoles. Occasionally, leeches and large fishes were caught in this manner. Dipping with nets was also employed in streams, where most animals were caught in the vegetation and debris associated with tree roots along the banks. Larger fishes were more effectively caught in seines, on trot lines and traps, or with bamboo fishing poles. Most leeches were caught on pieces of raw liver which were placed as bait near the shores of ponds. Snails were also collected by

hand picking them from the debris in the shallow margins of ponds.

Laboratory Maintenance of Hosts

Animals from field collections were kept alive in the laboratory until examined for parasites. The most widely used containers were 20-gallon aquaria filled with dechlorinated tap water which was filtered and aerated. Commercial catfish food (Purina) was suitable for most of the animals retained in the laboratory. Those large catfishes which were kept alive for experimental purposes were maintained in aerated children's plastic swimming pools, 5 feet in diameter by 1 foot deep. Leeches were kept in gallon jars which were provided with screened lids.

I attempted to lab rear several types of invertebrates, but was successful with only the snails, Helisoma trivolvis and Physa gyrina, and the dwarf crayfish, Cambarellus schufeldti. As an alternative to lab rearing such difficult animals as fresh-water shrimp, other species of crayfish, leeches, dragonfly naiads, and various fishes, I endeavored to find natural populations of each of these animals which were free of Alloglossidium and Macroderoides infections. I was able to accomplish this for all animals except the leech, Macrobdella ditetra.

Examination of Hosts for Parasites

Snails were examined for cercarial infections of Alloglossidium and Macroderoides. Each snail was isolated in a baby food jar filled with 50 to 100 ml of aged tap water and placed in darkness. Within two hours, if a snail was infected with Alloglossidium or Macroderoides, cercariae could be seen in the water with the aid of a dissecting microscope. Snails were routinely checked for cercarial emergence for several consecutive days. Some snails were examined for cercariae by dissection of the

internal tissues after the shell had been crushed. In some instances, snails which showed no indication of cercarial emergence were found to be infected. Generally, these infections were immature, but occasionally one such snail would contain active cercariae which appeared morphologically capable of emergence.

Insect larvae were macerated and examined for infections with a dissecting microscope. Crayfish were cut into two parts to expose the antennary glands. The glands were removed, examined for parasites and then the remainder of the crayfish was macerated and examined for parasites. Fresh-water shrimp were examined in the same manner, however, it was generally very easy to tell if the antennary glands contained A. renale because of the transparent exoskeleton of the shrimp. The fin rays of small fishes were first examined for parasites and then the body musculature was shredded and the peritoneal cavity was opened and examined. Tadpoles were examined in a similar manner. Leeches were stretched out and pinned for examination. A complete longitudinal incision of the body was made and followed by the longitudinal cutting of the crop, gastric ceca, and intestine. Adult trematodes were removed from the intestine with a pipette. Crop cysts and small coelomic trematodes were most easily seen after a few drops of AFA were applied to the leech's body. Only the stomach and intestine of large fishes were examined for trematodes. The gut was removed from the fish and opened in a dish containing 0.65% saline.

Examination of Parasites

The morphology of cercariae was most discernible when these organisms were alive. Examination of cercariae was made with phase contrast micro-

scopy at 970x. More detailed study of the internal structures such as penetration glands, ceca, caudal pockets, and rudiments of the genitalia was enhanced with the use of two vital stains, Neutral red, and Nile blue A. Cercariae were also killed and fixed for permanent preparations. The best results were obtained by killing the cercariae on a slide with a coverglass in place and passed over a flame for a few seconds. Just enough water was placed on the slide to keep the cercarial tail from doubling back on the body, but not to distort the cercaria with pressure. Cercariae killed in this manner were transferred to AFA for fixation.

Metacercariae were excysted with 00 insect pins with the aid of a dissecting microscope. The metacercariae were then fixed and stained in a manner similar to adult trematodes.

After removal from the host, adult trematodes were observed alive with phase contrast microscopy. The structure of the excretory bladder was better observed in living trematodes, but most trematodes were more readily studied when stained. Metacercariae and adults were fixed in a variety of ways. With the use of excessive coverslip pressure, trematodes could be flattened to the extent that normally obscured structural details could be studied. This method distorted these specimens so greatly, however, that they were not satisfactory for morphometric comparison. The method employed routinely for morphometric analysis was to place the trematode under slight coverglass pressure and to pipette cold AFA under the coverglass. Another method was to kill the adults in a manner similar to that described for cercariae. Finally, some trematodes were killed with immersion into 80°C water and then transferred to AFA. Morphometric comparison of trematodes fixed by each method was employed.

After fixation, trematodes were overstained in Semichon's carmine and destained with acid alcohol. The trematodes were then dehydrated with a graded series of alcohol, cleared and mounted on slides with Permount.

Serial sections of adult trematodes were made to study the structure of the excretory system and genitalia. Adult trematodes chosen for sectioning were killed by immersion in hot water to retain their normal body shape. Sections were made at 10μ and were affixed to microscope slides with either Mayer's albumin fixative or Haupt's medium. The latter gave the more satisfactory results. Slides containing the sections were stained with Harris' hematoxylin and eosin, and a coverslip was affixed with Permount.

Serial sections of shrimp infected with A. renale were made in a similar manner. The anterior portion of the cephalothorax was fixed in Bouin's fixative. Shrimp which had one antennary gland infected with A. renale and the other antennary gland uninfected were chosen for sectioning. Both transverse and sagittal sections were made of these shrimp.

Drawings of Alloglossidium were made with the aid of a microprojector and depict ventral views. All measurements are expressed in microns unless stated otherwise. Photographs of sectioned antennary glands were made with a Wild phase contrast microscope on Triatomic-X film.

Experimental Infections

Several methods were employed in an attempt to establish Alloglossidium infections in snails. Eggs of A. corti and A. renale following extrusion from the genital pore of the living trematodes were usually obtained from the bottoms of petri dishes containing distilled water. A. macrobdellensis and A. progeneticum did not freely extrude as many eggs, but

sufficient quantities of eggs were obtained from uteri of these two species with dissecting needles. Eggs were most often mixed with dried ground thistle and fed to laboratory reared Helisoma trivolvis or Physa gyrina. Several techniques to stimulate hatching of miracidia were attempted: exposure to sunlight, exposure to cold, incubation at 30°C, and mechanical agitation. Laboratory reared snails were also placed in aquaria with infected definitive hosts of each species of Alloglossidium.

The feces of snails were observed microscopically to determine whether hatching of the miracidia had occurred. Snails were placed in darkness to induce cercarial emergence between one and nine months after exposure to Alloglossidium. After repeated attempts to induce cercarial emergence, snails were crushed and examined microscopically for intramolluscan stages of Alloglossidium.

Animals that were exposed to cercariae generally were placed together in an aerated 1-gallon jar of dechlorinated tap water. Cercariae that had emerged from a naturally infected snail during the previous night were added to the gallon jar. Occasionally, an infected snail was added to the gallon jar of experimental animals. Animals exposed to cercariae in this manner were examined for infection, normally after two or three days, but sometimes one month or more following exposure to cercariae. Most animals used as experimental hosts were obtained from areas where natural infections do not occur. No uninfected leech, Macrobdella ditetra, could be obtained, but when leeches were exposed to A. macrobdellensis cercariae they became superinfected with many times the normal worm burden found in nature, as described by Corkum and Beckerdite (1975).

Catfishes which were collected from localities known to be free of

Alloglossidium infections were fed cysts of A. progeneticum with a pasteur pipette. Antennary glands of Procamabarus (Pennides) spiculifer which contained cysts of A. progeneticum were placed in the esophagus of catfishes with forceps. Catfishes were examined for adult A. progeneticum in the intestine from one to fourteen days following exposure to the cysts.

Catfishes from localities where Alloglossidium infections do not occur were fed A. renale. Some of these fishes were fed A. renale which had been dissected free of the antennary glands of fresh-water shrimp, Palaemonetes kadiakensis, while others were fed shrimp antennary glands containing A. renale in various stages of development. Another group of catfishes were placed in aquaria containing hundreds of shrimp infected with A. renale and were observed to eat every shrimp in the aquaria. Catfish intestine were examined for A. renale from one to fourteen days after exposure.

Screened cages were built to house experimental animals placed in Head of Island pond in Louisiana and Call's Creek in Georgia. One cage used at Head of Island was 3'x3'x2' and had a screen mesh of 1.5mm. It housed, at various times in 1974, University Lake fresh-water shrimp, and Procambarus (P.) spiculifer from Florida. One experiment at Head of Island was ruined by vandalism to the cage. Cages used at Head of Island thereafter were smaller (1'x6"x6") and had a larger screen mesh (4mm), and were completely submerged to avoid detection. These cages contained P. (P.) penni, P. (P.) vioscai, and Palaemonetes kadiakensis during experimental periods in 1975. Cages used at Call's Creek were constructed of gallon-sized plastic jars with 1.5mm mesh screen lids. These cages contained Procambarus clarkii, P. acutus acutus, P. (P.)

vioscai, and Palaemonetes kadiakensis from Louisiana and Procambarus (P.) spiculifer from East Athens Creek, Georgia. After experimental exposure to Alloglossidium cercariae, each of these hosts were examined for Alloglossidium infections in the laboratory.

Determination of Seasonality of A. renale

Collections of Palaemonetes kadiakensis at St. James and Head of Island, Louisiana were made over a period of 16 months, with an average interval of about three weeks between collections. Fifty to one hundred shrimp from each periodic collection were examined for infection with A. renale to determine if there was any seasonality in the incidence of A. renale in its host, P. kadiakensis. The number of A. renale in each antennary gland was noted, and whether the worms were immature, mature, or dead.

RESULTS

Morphological Comparison of Alloglossidium and Macroderoides

The collection and identification of macroderoidid trematodes constituted the initial phase of my study. In order to study the biology of Alloglossidium it was first necessary to determine whether this genus was morphologically and ecologically distinct from the closely related Macroderoides. With the exception of McMullen's (1935) work, no researcher has undertaken a comparative study of the two genera. McMullen (1935) reported the life cycle of Macroderoides typicus and added additional information to McCoy's (1928) life cycle study of Alloglossidium corti. McMullen separated Macroderoides and Alloglossidium on the basis of the structure of the excretory bladder and upon differences in host preferences. Yamaguti (1971) reviewed the generic diagnosis in Macro-

deroides by Pearse (1924) and the generic diagnosis of Alloglossidium by Simer (1929) and reported additional morphological differences between the two genera. The morphology of the specimens that I have examined from Louisiana confirms the reports of previous workers. I have found that Alloglossidium is separate and distinct morphologically from Macroderoides. Table I presents a list of the distinguishing characters of the two genera.

Based upon the accurate descriptions by McCoy (1928), McMullen (1935), Crawford (1937), Leigh (1958), and Corkum and Beckerdite (1975), I have observed that the cercariae of Macroderoides and Alloglossidium are easily distinguishable morphologically. This distinctiveness confirms the morphological separation of the two genera on the basis of larval as well as adult characters. These easily recognizable features have also allowed me to conduct experimental infections with the cercariae of both genera. Table II presents a list of characters by which cercariae of the two genera may be distinguished.

Morphological Comparison of the Species of Alloglossidium

A. corti (Figure 1) was collected from the following catfish hosts in southeastern Louisiana: Channel catfish, Ictalurus punctatus; Blue catfish, I. furcatus; Yellow bullhead, I. natalis; Brown bullhead, I. nebulosus; Black bullhead, I. melas; Tadpole madtom, Noturus gyrinus; and Black madtom, N. funebris. Anatomical data from these specimens were in agreement with descriptions of A. corti (Lamont, 1921; McCoy, 1928), and with a series of A. corti from Louisiana studied by Miller (1957). Similarly, a series of A. macrobdellensis (Figure 2) collected from Macrobdella ditetra in Louisiana corresponded closely to the des-

cription by Beckerdite and Corkum (1974). A. hirudicola does not occur in Louisiana. This trematode is structurally most similar to A. macrobdellensis, but Beckerdite and Corkum (1974) declared the two forms to be sufficiently different in morphology to be considered distinct species.

A series of sexually mature trematodes were collected from the antennary glands of the fresh-water shrimp, Palaemonetes kadiakensis in Louisiana. These trematodes could be identified as members of the genus Alloglossidium on the basis of the elongated I-shaped excretory bladder, the weakly developed cirrus sac, and vitellaria which extended anteriorly to the cecal bifurcation. Alloglossidium sp. from the fresh-water shrimp was, however, structurally distinct from either A. corti or A. macrobdellensis.

The species account of Macroderoides progeneticus from the antennary glands of the crayfish, Procambarus (Pennides) spiculifer, (Sullivan and Heard, 1969) indicated that M. progeneticus bore a close resemblance in both structure, host preference, and habitat to Alloglossidium sp. from the fresh-water shrimp. M. progeneticus does not occur in any locality where I have collected in Louisiana, but I compiled a topotypic series from P. (P.) spiculifer which I collected at Watkinsville, Georgia. M. progeneticus possesses an elongate, I-shaped excretory bladder, a weakly developed cirrus sac and preacetabular vitellaria, that are diagnostic of Alloglossidium. Sullivan and Heard described the elongate bladder, weak cirrus sac and preacetabular vitellaria in their species account, yet recommended that the generic diagnosis of Macroderoides be amended to accomodate their new species. Font and Corkum (1975) felt that this recommendation was unwarranted, since these characters are diagnostic of Alloglossidium. Therefore, it was proposed that M. progeneticus be as-

signed to the genus Alloglossidium on the basis of the structure of the bladder, cirrus sac and vitellaria.

Alloglossidium progeneticum (Sullivan and Heard, 1969) n. comb.

(Figure 3)

Hosts and Sites: Ictalurus nebulosus free in intestine

Procambarus (Pennides) spiculifer encysted in antennary gland

Locality: Call's Creek, Watkinsville, Oconee County, Georgia.

A. progeneticum most nearly resembles Alloglossidium sp. from the antennary glands of the fresh-water shrimp. The morphology of the shrimp trematode was compared to the description of A. progeneticum provided by Sullivan and Heard. Measurements of digenetic trematodes may be affected by different methods of fixation and slight differences in technique of individual researchers. To preclude this, I prepared a series of A. progeneticum and Alloglossidium sp. from shrimp in an identical fashion. Analysis of morphometric data from these two series and from the species account of Sullivan and Heard revealed that Alloglossidium sp. were both quantitatively and qualitatively distinctive in structure from A. progeneticum. Therefore, on the basis of these differences, Alloglossidium sp. from the fresh-water shrimp was described as a new species by Font and Corkum (1975).

Alloglossidium renale n. sp.

(Figure 4 & 5)

Description

Body ovoid 1.27-2.82 (1.88) mm long by .540-1.10 (.79) mm wide at midbody. Tegument finely spinous with spines becoming smaller and less numerous posteriorly but covering entire body. Mouth subterminal; oral

sucker 120-240 (150) by 100-220 (160). Acetabulum circular 90-180 (135) by 100-180 (135). Prepharynx short and undilated, 50-90 (70) by 50-110 (80). Esophagus short, cecal bifurcation close behind pharynx. Ceca dilated and thin-walled, terminating near posterior end of body. Testes diagonal, in posterior half of body. Anterior testis 80-180 (115) by 80-170 (120), posterior testis 100-190 (140) by 100-170 (135). Vasa efferentia enter cirrus sac separately. Cirrus sac 100-220 (160) by 30-80 (55), thin-walled seldom exceeding posterior margin of acetabulum. Cirrus small with internal bipartite seminal vesicle. Male genital pore median, immediately anterior to acetabulum. Ovary large, scalloped to lobate, 180-380 (265) by 200-365 (260) slightly dextral and dorsal, post acetabular. Mehlis' gland and Laurer's canal present, but inconspicuous. Seminal receptacle absent. Vitellaria follicular, laterally overlapping ceca dorsally and ventrally. Vitelline follicles extend from cecal bifurcation to level of posterior testis. Vitelline reservoir joins oviduct near ootype. Uterus highly convoluted at posterior end of body, completely filling body behind posterior testis. Ascending arm with several coils, only slightly inflated with eggs. More than two uterine loops passing between testes. Eggs contain viable miracidia when shed, 25-29 (28) by 13-16 (15). Uterine pore median, adjacent to male genital pore. Excretory bladder claviform, extending to posterior margin of ovary.

Type Host: Palaemonetes kadiakensis Rathbun, 1902.

Habitat: Antennary gland.

Type Locality: Mississippi River borrow pit, St. James, St. James Parish, Louisiana.

Type Specimens: U. S. N. M. Helm. Coll. Holotype 73780

2 Paratypes 73781

The most distinctive features by which A. renale can be separated from A. progeneticum are as follows. Living specimens of A. renale are more ovoid that the fusiform A. progeneticum. The size of the body and of individual organs is greater in A. renale with the exception of the prepharynx which is shorter and not dilated posteriorly. The cirrus sacs are about equally long, and in A. progeneticum it frequently overlaps the acetabulum posteriorly. The ceca of A. renale are typically broadly distended with ingesta. Although A. progeneticum is more active when removed from its host, very few eggs are extruded, but enormous numbers of eggs are shed by the more quiescent A. renale. The most striking morphological difference which can be used as an immediate recognition feature is the character of the uterus. The ascending arm of the uterus of A. progeneticum is sigmoid and greatly distended with eggs. In A. renale, the ascending arm is only slightly distended and makes several small turns between the level of the testes and the uterine pore. The differences between A. renale and A. progeneticum are summarized in Table III.

In summary, I have compared the adult structures of A. corti, A. macrobdellensis, A. progeneticum and A. renale. I am in agreement with the authors of these species that each species of Alloglossidium is morphologically distinct from other members of the genus. Furthermore, I accept the conclusions of Beckerdite and Corkum (1974) regarding the distinctiveness of A. hirudicola.

Ecological Relationship of Alloglossidium and Macroderoides

During the initial phase of collecting Alloglossidium and Macroderoides for morphological studies, I found that the two genera had distinctly different host preferences. The only vertebrate hosts found to harbor Alloglossidium in this study were catfishes, Ictalurus spp.

Macroderoides spp. adults were found in garfishes, Lepisosteus spp.; the Bowfin, Amia calva; and Pickerel, Esox americanus in Louisiana. Macroderoides has been reported occasionally from catfishes in other states. These reports may mean that catfishes represent an abnormal host (in the sense of Dogeil, 1964) for Macroderoides, or that due to structural similarities between the two genera, A. corti was misidentified. Miller (1957) found A. corti in catfishes but not in garfishes or bowfin in Louisiana. McMullen (1935) was unable to establish experimental infections of M. typicus in bullheads, Ictalurus sp.

Macroderoides metacercariae were found only in vertebrates in natural infections. Cysts were found in the tail musculature and fin rays of Mosquitofish, Gambusia affinis; Least killifish, Heterandria formosa; Golden topminnow, Fundulus chrysotus; and Sailfin molly, Poecilia latipinna and in the tail musculature and peritoneal cavity of tadpoles, Rana spp. Alloglossidium metacercariae were found in crayfishes, freshwater shrimp, and leeches, and Crawford (1937) has reported Alloglossidium metacercariae dragonfly, damselfly, and mayfly naiads in the northern United States.

No cysts of Alloglossidium have ever been reported from vertebrates. However, Sogandares (1965) reported Macroderoides typicus encysted in the thoracic musculature and antennary glands of crayfishes in Louisiana. Critical examination of his drawing indicates that he probably figured the metacercaria of A. corti.

Not only did the collection of life cycle stages of Macroderoides and Alloglossidium reveal that the two genera had widely divergent host preferences, but also it provided additional support for the generic reassignment of A. progeneticum. A. progeneticum was described from

crayfish, a normal host of Alloglossidium. In addition, I examined from the type locality of A. progeneticum in Watkinsville, Georgia, a catfish, I. nebulosus which was infected concurrently with A. corti and A. progeneticum. Unfortunately, this evidence for the involvement of the catfish in the cycle of A. progeneticum was not available to Sullivan and Heard. This evidence might have influenced the generic placement of their new species.

Although metacercariae and adults of Alloglossidium and Macroderoides have non-overlapping host preferences, the two genera utilize the same first intermediate host. For the life cycles which have been reported, the molluscan host is the planorbid snail, Helisoma. I have found Helisoma trivolvis in Louisiana infected with both Macroderoides and Alloglossidium cercariae. The incidence of Macroderoides infections was several times greater, and was also more widespread than the incidence of Alloglossidium.

Ecological Relationship of the Species of Alloglossidium

The species of Alloglossidium are separated by distinct structural differences. The taxonomy of the species of Alloglossidium is based solely on a morphological species concept. Populations within a species, however, often exhibit structural modifications which are dependent upon environmental factors. The populations which have these structural modifications are termed ecotypes. Since the host organism represents the immediate environment of a parasite, ecotypes, or host-induced variants are possible within a parasitic species which has broad host specificity. The classic works of Beaver (1937) and Rankin (1937) and the more recent work of Watertor (1967) have established host induced variation does occur in some species of digenetic trematodes. Because of hermaphroditism, and potential self fertilization of Alloglossidium, the usual test of

biological species, namely reproductive isolation, could not be applied in this study. The best alternative to the elucidation of the biological species of Alloglossidium was the examination of the ecological dependence or independence of the various morphological species of the genus. Ecological separation was measured by host preference, habitat selection, geographical isolation, seasonality of incidences and other differences in the life histories of the various species. Field studies of distribution and host preferences were supported by experimental infections attempted in the laboratory, and some controlled experimental infections attempted in the field.

A. Geographical Distribution

Collections were made in various habitats primarily in southeastern Louisiana, and to a lesser extent, in Georgia, Florida, and Alabama. Field collections revealed some information with regard to the range of the species of Alloglossidium and broad distributional patterns. More importantly, these studies determined the distribution of one species of Alloglossidium in relationship to the presence or absence of another (Table IV). The normal hosts of each species of Alloglossidium was ascertained and was compared with the distribution of each species. To understand the significance of these distributional accounts, one must bear in mind which combination of species of Alloglossidium are present in each locality, and which potential hosts for these species are present or absent. In this way, one may determine the possibility of ecological independence (= biological species) of two morphological species of Alloglossidium.

I shall present a hypothetical example to illustrate this method of determining ecological independence (Figure 6). Parasite A normally

utilizes host species 1. Parasite B normally utilizes host species 2. In pond X, host species 1 is present and infected with parasite A, and host species 2 is present and infected with parasite B. Two taxonomic possibilities exist. Either (1) parasites A and B are two valid biological species, or (2) parasites A and B are the same biological species and are only host induced morphological variants. If however, in Pond Y, host species 1 is present and infected with parasite A and host species 2 is present and uninfected (i.e. parasite B is absent) then it appears likely that parasites A and B represent ecologically independent, biological species. This conclusion is based upon the fact that, in this example, in the presence of suitable habitat (= host), the presence or absence of one parasite is independent of the presence or absence of the other.

1. Head of Island, Louisiana

A small pond with shallow margins lined with emergent vegetation provided one of the primary collection sites in this study. A. corti was present in the intestines of Yellow bullheads, I. natalis. Less than 25% of the bullheads were infected. The low infection rate at Head of Island was similar to the low rates found in most localities studied in southeastern Louisiana. A. corti metacercariae were found encysted in the antennary glands of only a few out of several hundred crayfish examined from this pond. The cysts were found most often in Procambarus acutus acutus, and once in P. hinei, but not in the abundant Cambarellus schufeldti. A. corti metacercariae have been reported from dragonfly, damselfly, and mayfly naiads (Crawford, 1937). I found none infected in my study, although these infections may have been overlooked due to the low rate of infections of the cysts in arthropods. In support of this

assumption, I have observed catfishes less than one inch in length which had gravid A. corti in their intestines. It appeared unlikely that fish this small had become infected through predation on crayfish. A. corti in catfishes ranged in development from newly acquired immature worms, to fully gravid and occasionally moribund forms. Metacercariae of A. corti in crayfishes varied in development from slightly larger than cercariae, with the slightest rudiments of ovary and testes, to worms which showed extensive gonadal development. However, egg production was not seen in A. corti metacercarial cysts. No Hellsoma trivolvis, of the several thousand examined for Alloglossidium infections, was found which was infected with A. corti, based upon data from experimental infections.

A. macrobdellensis was found in 100% of the leech, Macrobdella ditetra in the pond. Several H. trivolvis were found shedding A. macrobdellensis cercariae.

A. renale was found in the antennary glands of the fresh-water shrimp, Palaemonetes kadiakensis. The rate of infection in the shrimp varied seasonally from less than 10% to a maximum of 70%. Most of the life history studies of A. renale were done at Head of Island because of the large shrimp population and the high incidence of infection at certain times of the year. This pond was also the site of the most intensive search for the first intermediate host of A. renale. Although several thousands of molluscs were examined, none was found infected with A. renale based upon the inability of any cercariae in these molluscs to infect P. kadiakensis. No animals at Head of Island pond other than the fresh-water shrimp harbored A. renale infections. A. progeneticum was not found at Head of Island, nor any other location in Louisiana.

In summary, three species of Alloglossidium were found at Head of

Island pond: A. corti in crayfishes, Procambarus spp. and catfishes, Ictalurus spp.; A. macrobdellensis in the leech M. ditetra and the snail H. trivolvis, and A. renale in P. kadiakensis.

2. St. James, Louisiana

St. James pond is a borrow pit of the Mississippi River, and was subjected to large fluctuations in water level, including springtime inundation by the Mississippi River which occurred during two successive years during this study. Under typical conditions this pond was similar to Head of Island pond with emergent vegetation growing along the shallow margins. Toward the end of the study, the drastically reduced water level and silt from recent flooding killed the emergent vegetation and eliminated most of the invertebrate fauna of the pond.

A. corti does not occur in the St. James pond. Over 30 catfishes, I. natalis and I. punctatus ranging in size from less than 6 inches to over one foot and weighing in excess of three pounds, have been examined for parasites. None was infected with A. corti or with any other species of Alloglossidium. The same species of crayfishes were found here that occurred at Head of Island, but none was infected with any species of Alloglossidium. No species of Alloglossidium cercariae was found in H. trivolvis or any other mollusc.

A. macrobdellensis has not been found at St. James, but neither does its host, M. ditetra occur there. Two other leeches, Dina sp. and Philobdella gracilis were found in the wet mud under logs at the water's edge, but neither species was infected.

St. James pond is the type locality of A. renale from the freshwater shrimp, Palaemonetes kadiakensis. Rates of infection were very similar to those found at Head of Island, and data from both sites were

collected for life history studies. No snails were found infected with A. renale, nor were any other organisms except for the shrimp, infected with any life cycle stage of A. renale in St. James pond.

In summary, A. renale in P. kadiakensis was the only Alloglossidium at this locality, although the normal intermediate hosts and definitive hosts of A. corti were present.

3. Brusly, Louisiana

Whisky Bay is a small body of standing water, 20 yards wide by one-half mile long. It is mostly covered with floating and emergent vegetation.

A. corti was found in most of the catfishes, I. natalis, I. nebulosus, and Tadpole madtoms, Noturus gyrinus examined from this site. Several species of lentic crayfishes were present and Procambarus clarkii, P. acutus acutus, P. hinei, Orconectes lancifer and Cambarellus schufeldti were most abundant. These three Procambarus spp. and O. lancifer harbored A. corti encysted in the antennary glands, but the incidence of infection was extremely low.

Whisky Bay is the type locality of A. macrobdellensis. The population of its host, M. ditetra, is very large and virtually all of these leeches bear heavy infections of this parasite. Several Helisoma trivolvis have been found shedding A. macrobdellensis cercariae.

Two species of fresh-water shrimp occur at Whisky Bay, Palaemonetes kadiakensis and P. paludosus. The former shrimp is fairly easily collected, but the population is much smaller than those of St. James and Head of Island. P. paludosus is only occasionally collected at Whisky Bay. Neither shrimp has been found infected with A. renale at this locality.

In summary, A. corti and A. macrobdellensis occur at Whisky Bay, but A. renale does not, although its normal host inhabits this body of water.

4. Vacherie, Louisiana

Collections of Alloglossidium were made at the junction of Bayou Chevreuil and Highway 20. No catfish were caught here, but A. corti cysts were not found in over 100 crayfishes.

A. macrobdellensis occurs in M. ditetra and H. trivolvis.

A. renale occurs in P. kadiakensis collected from the bayou. The most important discovery made at this locality was one of over 100 Procambarus acutus acutus examined harbored six A. renale in an antennary gland. The worms had died recently, but death of A. renale is a commonly observed state in the normal shrimp host. The amount of decomposition was so slight that the structure of the worms was readily discernible. All six worms were fully mature. No cyst wall was present and the worms were morphologically identical to A. renale found in the antennary glands of fresh-water shrimp. I was able to determine that there was no variation in body structure or degree of maturation from being in an abnormal host.

5. Pierre Pass, Louisiana

Lake Verret is one of the largest lakes in Louisiana. Channel catfish, I. punctatus, and Yellow bullheads, I. natalis are abundant, and are commonly infected with A. corti. Sixty-seven percent of 45 catfishes were infected, typically with less than 5 A. corti, but one fish contained 42 gravid specimens.

M. ditetra, collected in the lake at a time when high water connected the lake with adjacent swamps, were infected with A. macrobdellensis.

Palaemonetes kadiakensis from the lake itself had no A. renale infections, but roadside canals near the lake contained infected shrimp.

6. Sorrento, Louisiana

The species composition of this locality was similar to both Head of Island pond and Lake Verret, in that all three species of Alloglossidium which occur in Louisiana were found there. I have collected in swamps and roadside ditches along Highway 61 and found Yellow bullheads, I. natalis, leeches, M. ditetra, and shrimp, Palaemonetes kadiakensis infected with A. corti, A. macrobdellensis and A. renale respectively.

7. Shreveport, Louisiana

Palaemonetes kadiakensis from Caddo Lake, collected by Mr. George J. Greer were infected with A. renale. This collection represents the northernmost point of the known distribution of A. renale. I have no data on any other Alloglossidium from this locality.

8. Bogalusa, Louisiana

A. corti metacercariae were found in a high percentage of crayfishes from Lee's Creek. Infected crayfishes were Orconectes lancifer, Procambarus acutus acutus, and P. (Pennides) penni. The latter species is, according to Hobbs (1962) the most closely related crayfish species to P. (P.) spiculifer, the type host for A. progeneticum in Georgia. One A. corti metacercaria was found in the antennary gland of each of two Palaemonetes kadiakensis. Despite being in an abnormal host, these specimens of A. corti were morphologically indistinguishable from A. corti from crayfish, including the presence of the normal cyst wall in the antennary gland, and the total absence of eggs in the uterus.

Leeches, which are the only hosts of A. macrobdellensis were not collected in Lee's Creek.

A. renale infections were found in several Palaemonetes kadiakensis from this creek. No crayfish, including Procambarus (P.) penni were infected with A. renale.

9. Laurel Hill, Louisiana

One A. corti metacercaria was encysted in one of over 100 Palaemonetes kadiakensis from Lake Rosemound. The specimen was structurally similar to A. corti metacercariae from normal hosts. Lake Rosemound is the only locality except for Lee's Creek where A. corti has been found in fresh-water shrimp.

10. Baton Rouge, Louisiana

University Lake is unique in that it is the only body of water in Louisiana in which, after extensive collections were made, I have not found any Alloglossidium infections. I have examined the following hosts: over 100 crayfishes, Procambarus clarkii, and P. a. acutus, several thousand shrimp, Palaemonetes kadiakensis, and over 20 catfishes, I. punctatus and I. natalis. The leech, M. ditetra, did not occur in University Lake.

These Alloglossidium free animals were extensively used for experimental infections in the laboratory.

Fresh-water shrimp from this lake became infected with A. renale when placed in cages in ponds where natural A. renale infections occur, indicating that University Lake shrimp possess no innate resistance to infection.

11. Other Louisiana Localities

I have collected, or attempted to collect Alloglossidium from many other aquatic environments in the state. I have listed each of these locations under Materials and Methods. While none of these warrant a sep-

arate discussion, nonetheless each is significant in that it either confirmed observations made at the abovementioned localities, or in some way aided in the elucidation of the relationships of the species of Alloglossidium and their hosts.

12. Watkinsville, Georgia

I collected Procambarus (P.) spiculifer from Call's Creek to obtain topotypic series of A. progeneticum. One hundred percent of the crayfish were infected with cysts of A. progeneticum. Most crayfish had several cysts in the antennary glands, but some crayfish had as many as 150 cysts. In heavy infections, the antennary glands were filled with cysts. There was a decreasing gradient in the number of cysts found in each part of the body until at the posterior end of the abdomen, only a few A. progeneticum had encysted in the musculature. The cysts in any one crayfish ranged in development from cercaria size, with slight organogenesis and the shouldered stylet free in the cyst, to fully mature worms with a strikingly distinct S-shaped uterus broadly distended with eggs, and finally to dead worms and the egg mass remnants of decomposed A. progeneticum. The worm burden was in general, directly related to crayfish size. Even the smallest juveniles were infected, often with gravid worms.

I collected in Call's Creek, one Brown bullhead, I. nebulosus, which was infected with both A. progeneticum and A. corti. The eleven specimens of A. progeneticum were free in the intestine. Except for the lack of a cyst wall, they were identical to A. progeneticum from P. (P.) spiculifer. The four specimens of A. corti were free, and found in the same region of the intestine as A. progeneticum. A. corti from this bullhead were identical to specimens of A. corti from catfishes in Louisiana.

The two species were so distinct that I was able to identify them even under the relatively low power (30x) of a dissecting microscope.

I did not find A. corti metacercariae in crayfish from Call's Creek. The number of A. progeneticum in these hosts was so great that it is probable that I could have overlooked lighter infections of A. corti.

No leeches were collected in Call's Creek and I do not know if A. macrobdellensis was present.

Palaemonetes kadiakensis does not occur in Call's Creek, or within at least a 100 mile radius of Watkinsville, Georgia. No specimens resembling A. renale were found in any host in Call's Creek, or in any other locality in Georgia.

13. Other Georgia Localities

I have collected A. progeneticum in P. (P.) spiculifer from the Middle Oconee River and Call's Creek, which flows into the Middle Oconee. Crayfish from the headwaters of the Middle Oconee at I-85 had a low incidence of infection. I found higher infections in P. (P.) spiculifer from the Middle Oconee in Athens, Georgia, but the number of cysts per host was far lower than the collections from Call's Creek. No P. (P.) spiculifer or P. (P.) raneyi, a closely related species, harbored A. progeneticum, even those from streams within a few miles of the type locality.

Palaemonetes kadiakensis from McKinney's pond and mill race at Midville, Georgia were not infected with Alloglossidium. Procambarus (P.) spiculifer was not present at this locality, but the Procamabarus sp. in both the pond and the stream was not infected with Alloglossidium.

Mr. Richard W. Heard III has provided me with specimens of Alloglossidium from the antennary glands of Orconectes clypeata near Irwinton, Georgia. These specimens resemble A. progeneticum except that the uterus contains very few eggs and (according to Mr. Heard)

some specimens were not encysted. He has also given me specimens of A. corti metacercariae from Georgia crayfish, which I was unable to collect in my own field studies in Georgia.

14. Florida and Alabama Localities

Two crayfish of the subgenus Pennides were examined for Alloglossidium at several localities in southern Alabama and northwestern Florida. A. corti metacercariae were common parasites in the antennary glands of both species, P. (P.) spiculifer and P. (P.) versutus, but A. progeneticum was not found. Palaemonetes kadiakensis was abundant at Sink Creek, Florida where many A. corti metacercariae were found in P. (P.) spiculifer. The shrimp, however, were not infected with any species of Alloglossidium.

One large leech, Macrobdella ditetra was collected near Pensacola, Florida. The leech was not infected with Alloglossidium and is the only specimen of its size that I have found uninfected.

Summary of the Geographical Distribution

A. corti, A. macrobdellensis, and A. renale, but not A. progeneticum have been found in Louisiana. These first three species have been collected at the same locality, but each has also been found in other localities independent of the presence of the other two. A. corti and A. macrobdellensis have been found in ponds where A. renale did not occur, although the shrimp host of A. renale was present. A. renale has been found in a pond where neither A. corti or A. macrobdellensis occurred, although the catfish and crayfish hosts of A. corti were present.

A. progeneticum has been found only in Georgia. It has been found in a catfish concurrently infected with A. corti. A. progeneticum was not found in P. (P.) penni, the nearest relative of the type host, P.

(P.) spiculifer, in Louisiana streams which contained fresh-water shrimp infected with A. renale.

B. Experimental Infections

1. Infection of Laboratory Reared Snails

I attempted to establish the intramolluscan stages of each species of Alloglossidium in laboratory reared snails for several reasons. The primary reason was to expose the cercariae derived from these infections to each of the natural hosts of Macroderoides and Alloglossidium. From these cercarial infections I could then determine the host specificity of each species of Alloglossidium, and in those instances that two or more different hosts were infected, I could measure any host induced morphological effects. Infection of laboratory reared snails would also have made possible the description of the intramolluscan stages and a determination of the first intermediate host specificity of each species of Alloglossidium.

I was totally unsuccessful in establishing infections in laboratory reared snails. Several other workers have attempted similar infections and failed. The natural snail host for A. corti and A. macrobdellensis is Helsoma trivolvis (McCoy, 1928; McMullen, 1935; Corkum and Beckerdite, 1975). Most attempts to infect snails by the methods described under Materials and Methods involved laboratory reared Helisoma trivolvis. Over one thousand H. trivolvis, from newly hatched to one year old snails were refractory to infection with A. renale, A. corti, A. macrobdellensis, and A. progeneticum. Experimental infection of several hundred laboratory reared Physa gyrina and wild Physa sp. from Call's Creek were also unsuccessful. Numerous Alloglossidium eggs were found in the feces of Helisoma and Physa with the opercula removed, and a few dead

miracidia were found. No sporocysts developed in these snails despite the apparent hatching of the miracidia.

2. Cercarial Infections from Naturally Infected Snails

Since no cercariae could be reared in the laboratory, I used Helisoma trivolvis naturally infected with Macroderoides and Alloglossidium cercariae to infect the hosts of these two genera. Macroderoides sp. cercariae were the most common trematode parasites of H. trivolvis. Many H. trivolvis infected with Macroderoides were placed with the following hosts which were collected from localities known to be free of natural Macroderoides and Alloglossidium infections: tadpoles, Rana spp. mosquitofish, Gambusia affinis, Least killifish, Heterandria formosa, several kinds of crayfishes, Procambarus spp., Orconectes spp., and Cambarellus schufeldti, fresh-water shrimp, Palaemonetes kadiakensis, leeches Macrobdella ditetra, Placobdella sp., Helobdella sp. and unidentified damselfly, dragonfly and mayfly naiads. Macroderoides cercariae almost always infected the tadpoles and fishes. Numerous cysts were found in the somatic muscles and peritoneal cavity of tadpoles. Encystment most often occurred in the fins of small fishes, but some cysts were found in the muscles and body cavity as well. No other hosts became infected with Macroderoides, with one exception. Occasionally crayfishes, Procambarus acutus acutus and Cambarellus schufeldti, when exposed to massive numbers of Macroderoides cercariae, would become infected. As many as thirty cysts were found in an infected crayfish, always in the thoracic muscles at the bases of the periopods. These cysts were able to survive for at least one month in the laboratory. However, I have not found natural infections of Macroderoides in crayfishes.

Helisoma trivolvis shedding Alloglossidium cercariae were also found

on numerous occasions, but the incidence of infection was much lower than that of Macroderoides. From the experimental infections that I conducted, I was able to determine that every one of these infected snails was shedding A. macrobdellensis cercariae. The cercariae were exposed to the same hosts as those listed above for Macroderoides. The only host which was infected with these cercariae was Macrobdella ditetra. Typically, these experimental leeches would contain several hundred unencysted A. macrobdellensis in the coelom which were identical to those from experimental infections described by Corkum and Beckerdite (1975). A few crayfish and fresh-water shrimp molted during the infection period, but molting did not enhance the infection of these hosts.

An extensive search for the cercariae of A. corti and A. renale in H. trivolvis in Louisiana was unsuccessful. This search was concentrated primarily on an examination of H. trivolvis since this snail is the only known host for A. corti and had the greatest potential for being the first intermediate host of A. renale. Several thousand Helisoma were examined from aquatic habitats in which there was a high incidence of A. corti in catfishes. Several thousand Physa gyrina and a lesser number of Lymnea sp., Viviparus sp., Gyralus sp., and Sphaerium sp. were also examined for Alloglossidium infections. None of these molluscs shed cercariae closely resembling the cercariae of Alloglossidium. However, any cercariae which had some anatomical similarities with Alloglossidium were placed with the abovementioned hosts. No Alloglossidium infections resulted from these attempts at experimental infection.

Physa sp. from the type locality of A. progeneticum was examined for cercariae. Only one type of cercaria was found. The stylet of this cercaria resembled the shouldered stylet of Alloglossidium, but the bladder

was distinctly Y-shaped. No experimental infections occurred in hosts exposed to this cercaria.

In summary, Macroderoides generally infects only tadpole and fish hosts. The only Alloglossidium cercaria found was A. macrobdellensis. It's host specificity was restricted solely to Macrobdella ditetra.

3. Experimental Infections at Head of Island, Louisiana - 1974

During the spring, 1974, uninfected Palaemonetes kadiakensis from University Lake, Baton Rouge, Louisiana were placed in a screened cage at Head of Island pond. I attempted to infect the shrimp in this manner to ascertain whether University Lake shrimp were uninfected in their natural habitat because of an innate resistance to A. renale present in the population. The practical application of these results was that if the University Lake population was resistant to infection, then it was not suitable for use as experimental hosts in experimental infections attempted in the laboratory. However, the most important reason for this experiment was to determine the rate of maturation of A. renale in its shrimp host. The University Lake shrimp did become infected with A. renale at Head of Island, which established that the University Lake population is not innately resistant to infection. The results of the study of the rate of maturation of A. renale will be presented under another section.

In the summer, 1974, Procambarus (P.) spiculifer from the Fish River in Alabama were placed, along with University Lake fresh-water shrimp as controls, in the same screened cage at Head of Island. No infections resulted in either the shrimp or crayfish. Other research on the seasonal cycle of A. renale showed that the lack of infections in these experimental hosts was due to the cessation of cercarial emergence of A. renale during this period. This experiment did establish, however, that

a crayfish of the subgenus Pennides, which inhabits a lotic environment, could survive in a lentic habitat under the experimental conditions. This preliminary information meant that the experiment could be repeated in the following year during periods of emergence of A. renale cercariae.

4. Experimental Infections at Head of Island, Louisiana - 1975

A second attempt was made to infect crayfish of the subgenus Pennides with A. renale at Head of Island pond in January and February 1975. Procambarus (Pennides) penni were collected from Talisheek Creek, Talisheek, Louisiana. Over 50 specimens of P. (P.) penni were examined in the laboratory and were found to be free of any Alloglossidium infections. Similarly, over 50 P. (P.) vioscai from Hog Branch, near Livingston, Louisiana were examined for infections of Alloglossidium. Several were infected with cysts of A. corti in the antennary glands, but harbored no other species of Alloglossidium. Twenty specimens of P. (P.) penni and 20 P. (P.) vioscai were placed in screened cages in Head of Island pond in January. Over 100 uninfected University Lake fresh-water shrimp were also placed in the pond as controls for the experiment. Simultaneously, a collection of Palaemonetes kadiakensis from Head of Island was examined for A. renale infections. Over 30% of the shrimp were infected with A. renale, and the appearance of new A. renale infections indicated that A. renale cercariae, emerging from the first intermediate host, were present in the pond at that time.

After one month, all experimental animals were returned to the laboratory and examined for Alloglossidium infections. None of the 20 P. (P.) penni was infected with any species of Alloglossidium. Of the 20 P. (P.) vioscai, three specimens harbored one A. corti cyst in the antennary gland. Each A. corti cyst was large enough to indicate that

it was due to an old infection, probably acquired at Hog Branch. None of the crayfishes had any A. renale infections. Of the fresh-water shrimp placed in the screened cage, all but six had died, and none of these six had become infected. Because of the lack of adequate controls, it is possible that the crayfish were not exposed to A. renale cercariae. The infection rate of A. renale in the natural population of fresh-water shrimp at Head of Island, however, had risen during the one month experimental period. This rise in infection indicated that exposure of A. renale cercariae to the crayfishes did occur, but that these crayfishes of the subgenus Pennides were not suitable hosts for A. renale.

5. Experimental Infections at Watkinsville, Georgia

Fresh-water shrimp and Procambarus (Pennides) vioscai, from Louisiana were placed in cages in Call's Creek for three days. Uninfected P. (P.) spiculifer from nearby East Athens Creek served as controls, and were placed in containers with the Louisiana hosts. At the end of this brief experimental period, examination of all experimental hosts revealed no Alloglossidium infections. P. (P.) spiculifer collected from Call's Creek at the same time had infections of cercaria sized A. progeneticum, indicating that cercarial emergence was taking place during the experimental period. It appears that either three days and/or the size of the cages did not allow sufficient exposure of the hosts to A. progeneticum cercariae.

6. Experimental Infections of Catfishes in the Laboratory

Several attempts were made to infect catfishes with A. renale. Uninfected catfishes from University Lake were fed all developmental stages of A. renale. A. renale dissected from Palaemonetes kadiakensis and A. renale which remained intact within the antennary glands were placed in

the gut of fishes by means of a stomach tube. In addition, uninfected catfishes were allowed to eat many shrimp infected with A. renale. No A. renale infections could be established in catfishes, although examination of the gut contents showed that infected shrimp had been eaten by the fishes.

I have placed A. progeneticum cysts in the gut of two catfishes by gavage but was not able to infect either fish. I have observed that in previous experimental infections with A. renale, catfishes often regurgitated the parasites when they were placed in the fishes' guts with the stomach tube. It is possible that the cysts of A. progeneticum were expelled in a similar manner.

C. Ecological Relationship of Alloglossidium renale and Palaemonetes kadiakensis

A. renale from the fresh-water shrimp was undescribed at the onset of this study. Therefore, as a major part of this research on the genus Alloglossidium, I attempted to elucidate various aspects of the life history of A. renale. Corkum and Beckerdite (1975) established that A. macrobdellensis has an annual life cycle that is directly linked to that of its definitive host, Macrobdella ditetra. I attempted to determine if A. renale has a similar host related cycle in P. kadiakensis, and to ascertain the cause of any seasonality in the infection. I selected a Mississippi River borrow pit at St. James, Louisiana as my study area because of its large shrimp population and the high percentage of A. renale infections. When impending flood conditions threatened the St. James pond early in 1974, I established a second study site in a small pond at Head of Island, Louisiana because of the more stable habitat which it provided.

I observed a pronounced annual cycle in the incidence of Alloglossidium renale in the antennary gland of the fresh-water shrimp (Figures 7 & 8). New infections of A. renale first appeared in shrimp in early autumn. The youngest worms were slightly larger than Alloglossidium cercariae and possessed remnants of the stylet and penetration glands. Cercarial emergence increased throughout the winter and spring, so that the total percentage of infected shrimp rose from 10% in October to 70% in May. New infections of A. renale ceased in May, and no immature worms were found from June through September. An interval of one to two months was observed between the increase of new infections in late autumn and the rise of mature infections in early winter. Several small peaks occurred in the percentage of shrimp infected with immature A. renale. These peaks presumably were caused by increases in the cercarial emergence from the first intermediate host. From one to two months following these peaks, there was a corresponding increase in the percentage of shrimp infected with gravid worms. The lag time between the appearance of new infections and the increase in mature infections therefore, probably represented the time required for maturation of A. renale in its definitive host.

Experimental infections were used to confirm this supposition. We placed uninfected shrimp from University Lake, Baton Rouge, Louisiana in a screened cage at Head of Island pond. Shrimp infected in this manner were examined in the laboratory. Slight development of the testes was present in even the youngest worms. Growth of A. renale occurred in the antennary gland without encystment and worms were fully gravid in six weeks.

Mature specimens of A. renale produced enormous numbers of eggs which escaped from the host through the excretory pore of the antennary

gland. In some of the largest worms the uterus was partially depleted of eggs and occasionally, moribund worms were observed. Dead worms were found in the antennary glands of shrimp through the year. Infrequently, these worms appeared to be recently dead, but more often some decomposition had taken place. In the most extreme condition, the only evidence of infection was a cluster of A. renale eggs in the antennary gland. The percentage of shrimp infected with dead worms was fairly constant except in late spring. In April the number of dead worms increased sharply and remained at its highest level until June. Most dead worms observed during the late spring appeared to have died recently, whereas at other times of the year badly decomposed worms were most often observed. The data from Figures 7 & 8 indicate that most specimens of A. renale had a short life span. Shrimp harboring dead A. renale in late spring must have become infected within the previous six months since the percentage of shrimp with dead worms exceeded the total percentage of infected shrimp prior to the onset of new infections in October. Dead worms were sometimes found in multiple infections with living gravid and immature specimens. The mixed stages of development in multiple infections were an indication that shrimp offered no resistance to reinfection with A. renale. Most multiple infections occurred in late spring when both the highest numbers of worms per host and the maximum percentage of infected shrimp were reached.

After the cessation of new infections in May, the percentage of infected shrimp fell to less than 10% until new infections reappeared in the following October. Population dynamics of A. renale were similar in the two ponds during most of the study period (compare Figures 7 & 8) even though St. James pond was subjected to flooding by the Mississippi River during the spring of 1974. In the summer and autumn of 1974, how-

ever, the drastically lowered water levels and the accumulation of silt at St. James pond caused the destruction of all emergent vegetation and then the virtual elimination of all snails from the pond. Therefore no new infections of A. renale in the fresh-water shrimp occurred at St. James in autumn 1974, while new infections at Head of Island reestablished the annual cycle of A. renale at that locality.

Vertebrates had no role in the seasonal population dynamics of A. renale in this study. Unlike A. corti which uses catfishes as a definitive host, and A. progeneticum which has been reported from a catfish as well as a crayfish (Font and Corkum, 1975), A. renale has not been found in over 200 catfishes, including 30 examined from St. James and Head of Island. Parasite-free catfishes were fed shrimp infected with A. renale. In no case could an infection be established in a catfish.

I have determined from laboratory observation of shrimp infected with A. renale, that egg dispersal occurs without any involvement of vertebrates. I have observed eggs of A. renale in the labyrinth of the excretory gland, and distally throughout the entire excretory tubule to the rim of the excretory pore itself. Eggs containing active miracidia have been found in the bottoms of containers in which infected fresh-water shrimp were kept.

The presence of A. renale in the excretory gland produces a gross distention of the labyrinth (compare Figures 9 and 10). There is also mechanical erosion of the tissue of the labyrinth and saccule by the tegumental spines of A. renale (Figure 11), but in my study, the pathology of A. renale infections had no discernible effect on either individual shrimp, or on the entire host population.

D. Seasonal Incidence of Other Species of Alloglossidium

The only other detailed study of the seasonal incidence of Alloglossidium infections was conducted by Corkum and Beckerdite (1975) for A. macrobdellensis. In their study, Macrobdella ditetra was infected continuously throughout the year with cercariae of A. macrobdellensis. Leeches that I have examined in my study harbored new infections in every season of the year. I have found Helisoma trivolvis shedding A. macrobdellensis cercariae in all four seasons.

Because of the low incidence of infection of crayfishes with A. corti metacercariae, I have not been able to determine whether any seasonal incidence of infection exists for A. corti in Louisiana. I have found catfishes infected with A. corti in all seasons of the year, but since these infections do not arise from cercarial penetration, as do infections of M. ditetra and Palaemonetes kadiakensis, the seasonality of these infections cannot be compared. Crawford (1937) reported that A. corti cercariae did not emerge from Helisoma trivolvis in the summer. Mature infections developed in the fall and persisted throughout the winter and spring. In contrast to Crawford's data, Cort, McMullen and Brackett (1939) found that the summer incidence of cercaria which they identified as A. corti from Helisoma campanulatum reached as high as 71% of the large snails examined. The discrepancy between the two studies may be explained by the fact that both were conducted when Alloglossidium was a monotypic genus. Either study may have been influenced by the presence of cercariae of an undescribed species of Alloglossidium.

I collected A. progeneticum only in May and August in Georgia, and did not study the seasonal incidence of infection. In both months, however, I found new infections representing recent cercarial penetrations. Therefore, if the cercarial emergence of A. progeneticum in Georgia is

seasonal, it nevertheless is not in synchrony with the seasonal emergence of A. renale cercariae in Louisiana.

DISCUSSION

Causation of the Annual Cycle of Alloglossidium renale

Several factors were responsible for the annual cycle of the incidence of A. renale infections in the fresh-water shrimp, Palaemonetes kadiakensis. The increase in the total percentage of infected shrimp occurred gradually over an eight month period. The slow rise in incidence was caused solely by the acquisition of new infections in fresh-water shrimp due to cercarial penetration. The emergence of cercariae from the first intermediate host first began in October and continued at a slow rate throughout late fall and winter. Cercarial emergence increased greatly in the spring until the end of May. In contrast, the decline in the percentage of A. renale infections occurred precipitously within six weeks for three reasons. First was the cessation of new shrimp infections in June, which meant that cercariae were no longer released from the first intermediate host. Second was the death of many A. renale after maturation and a period of prolific egg production. The third and probably the most significant reason was the death in early summer of the generation of shrimp which had become infected in the previous eight months. Seasonal mortality in fresh-water shrimp was first reported by Meehan (1936) who observed that the parent generation of shrimp which bred in the spring was replaced by its offspring in the summer in Louisiana. I have observed a similar seasonal mortality in both infected and uninfected shrimp populations.

Corkum and Beckerdite (1975) reported the seasonal incidence of A. macrobdellensis in the leech, Macrobdella ditetra. The seasonal inci-

dence of A. renale in Palaemonetes kadiakensis is similar to that of A. macrobdellensis in that the incidence is influenced by the life span of the definitive host. One difference, however, is that new infections are continuous throughout the year in A. macrobdellensis, whereas in A. renale cercarial emergence does not occur in June through September and is at a maximum in the spring. The annual cycle of A. renale closely parallels the life cycle of its definitive host, Palaemonetes kadiakensis. Although most infections occur near the end of the life span of the freshwater shrimp, A. renale matures rapidly, produces many eggs during a short period, and dies shortly before the death of its host.

Morphological Comparison of the Species of Alloglossidium

The genus Alloglossidium is composed of five species, A. corti, A. progeneticum, A. renale, A. hirudicola, and A. macrobdellensis, which are morphologically distinct and separate from each other. The genus can, however, be divided into two groups on the basis of the morphological similarity of certain of the species. The two species A. hirudicola and A. macrobdellensis that occur in leeches can be placed into one group because of their elongated body form, spherical ovary, tandem testes, ceca which terminate short of the posterior extremity and the similarity of the measurement of the body size, suckers, and gonads. The second morphological group of Alloglossidium is composed of A. corti, A. progeneticum, and A. renale which parasitize arthropods. These three species of Alloglossidium are fusiform or ovoid in outline, and are never elongated. A. corti, A. progeneticum, and A. renale possess a distinctly lobed ovary, tandem testes, and ceca which terminate near the posterior extremity. Within this second group of species of Alloglossidium, A. progeneticum bears a closer resemblance to A. renale than to A. corti in

that A. progeneticum and A. renale have testes which are smaller than the ovary and both possess only fine tegumental spination.

Ecological Comparison of the Species of Alloglossidium

In addition to being morphologically distinct from one another, each species of Alloglossidium is ecologically independent.

The only species of Alloglossidium collected from St. James pond was A. renale. A. renale was found, at certain times of the year, in a large percentage of the fresh-water shrimp at St. James, but was absent from crayfishes and catfishes. If A. renale and A. corti were in actuality, ecotypes of the same species of Alloglossidium, then one would expect to find the crayfishes at St. James infected with Alloglossidium unless some other ecological factor prevented the crayfishes from becoming infected. However, no ecological barrier of this nature exists since crayfishes, fresh-water shrimp and snails all occur together in the emergent vegetation of the pond. Because Alloglossidium cercariae emerge from a snail, and swim about randomly until they contact a potential host, there is a large probability that encounters of cercariae with both shrimp and crayfishes occur. The fact that A. renale occurs in the fresh-water shrimp, but no Alloglossidium occur in the crayfishes in St. James pond demonstrates that A. renale is a species that is ecologically independent of A. corti.

Additional evidence supports the conclusion of the independence of A. renale and A. corti. Catfishes at St. James pond are not infected with Alloglossidium. Fresh-water shrimp are a common source of food for catfishes, and have been found in the intestines of catfishes examined from St. James. If A. renale and A. corti were ecotypes, then catfishes should become infected by ingesting shrimp harboring Alloglossidium.

Since catfishes do not become infected in this manner, either at St. James pond, or under laboratory experimental conditions, then I conclude that A. renale and A. corti are separate species which have mutual ecological independence.

The species composition of Alloglossidium in its hosts at Whisky Bay in Brusly, Louisiana also illustrates the independence of A. corti and A. renale. At Whisky Bay, both crayfishes and catfishes are parasitized by A. corti, but fresh-water shrimp which are common in this body of water do not harbor Alloglossidium. The potential for cercarial penetration of the shrimp is present in this locality, but the lack of shrimp infections must mean that A. corti cercariae do not normally possess any specificity for the fresh-water shrimp.

Another conclusion regarding the biological species of Alloglossidium can be drawn from the species composition at Whisky Bay. Virtually 100% of the leeches, Macrobdella ditetra, are infected with A. macrobdellensis here. The same potential exists for cercarial penetration of fresh-water shrimp as for infection of leeches, but no Alloglossidium infections are found in the shrimp. A. macrobdellensis and A. renale are therefore ecologically independent and are two distinct biological species.

The concurrent infection of a specimen of the Brown bullhead, Ictalurus nebulosus, from Georgia, with both A. corti and A. progeneticum indicates that these two species of Alloglossidium cannot be host induced morphological variants. The specimens of both A. corti and A. progeneticum from the intestine of the bullhead were easily identifiable and were similar structurally to specimens of A. corti and A. progeneticum collected from other hosts. A. corti and A. progeneticum were independent of one another in localities that I studied in Florida and Alabama. Many Procambarus (Pennides) spiculifer and P. (P.) versutus from these

sites had A. corti cysts, but none was infected with A. progeneticum.

Evidence from collections made at Lee's Creek in Louisiana also supports the conclusion that A. corti and A. progeneticum are distinct biological species. In this creek, crayfishes including P. (P.) penni, are heavily infected with A. corti metacercariae encysted in the antennary glands, but are not infected with A. progeneticum. The only known crayfish host for A. progeneticum is P. (P.) spiculifer, but this species of crayfish does not occur in Louisiana. Other members of the subgenus Pennides that do occur in Louisiana are potential hosts of A. progeneticum however, I have not found A. progeneticum in any P. (Pennides) sp. crayfish in Louisiana, including P. (P.) penni at Lee's Creek.

Lee's Creek is also a source of evidence that A. renale and A. progeneticum are ecologically independent. I have examined fresh-water shrimp from Lee's Creek which were infected with A. renale, but P. (P.) penni from the same locality was not parasitized by either A. renale or A. progeneticum. One may argue that the host specificity of A. progeneticum may not include P. (P.) penni. It is highly improbable, however, that if the specificity of A. progeneticum does not include P. (P.) penni, which according to Hobbs (1962) is the closest relative of P. (P.) spiculifer, that A. progeneticum would be specific for the very distantly related fresh-water shrimp, Palaemonetes kadiakensis. I have concluded that A. renale and A. progeneticum are ecologically, as well as morphologically separate, and are therefore, distinct biological species.

In the determination of the ecological independence of A. macrobdellensis, I encountered a unique problem. I have been unable to find any locality in Louisiana which had a population of the leech, Macrobdella ditetra, which was not infected with A. macrobdellensis. In only

one instance have I been able to determine the independence of A. macrobdellensis from another species of Alloglossidium. As I have reported above, A. macrobdellensis is ecologically independent of A. renale because at Whisky Bay, A. macrobdellensis is present but fresh-water shrimp are not infected with Alloglossidium. Experimental infections in the laboratory, however, confirm that A. macrobdellensis is a distinct biological species. Many Helisoma trivolvis infected with Alloglossidium cercariae were placed with all the known hosts of Alloglossidium. In every instance, no host became infected except M. ditetra. These leeches had massive infections identical to the infections described by Corkum and Beckerdite (1975). The results of the experimental infections indicated that A. macrobdellensis cannot infect hosts which normally harbor other species of Alloglossidium, and therefore, A. macrobdellensis is a separate biological species.

Of the several thousands of hosts of Alloglossidium which I have examined, I have only found four instances in which one species of Alloglossidium parasitized the normal host of a different species of Alloglossidium. Specifically, I found one crayfish infected with six specimens of A. renale and three fresh-water shrimp each infected with one specimen of A. corti. The A. renale in the crayfish were structurally similar to the specimens of A. renale from fresh-water shrimp, which included the lack of cyst walls in the antennary gland of the crayfish. The specimens of A. corti in the fresh-water shrimp were structurally similar to specimens of A. corti from crayfish, which included the presence of a normal cyst wall. Therefore, when the normal host specificity of A. corti and A. renale breaks down, development of the parasite occurs normally, without any host induced variation.

In summary, I have concluded that on the basis of field and labora-

tory studies, A. corti, A. progeneticum, A. renale, and A. macrobdellensis are morphologically and ecologically distinct from each other and are therefore separate biological species. Figure 12 depicts the ecological relationships of the species of Alloglissidium. A key to the species of Alloglossidium is presented in Table V.

The Species Problem in Digenetic Trematodes

The primary species concept of helminthologists historically had as its basis a morphologically defined species. The importance of adult structures was also paramount in the first attempts made to deduce phylogenetic relationships of digenetic trematodes. Hence, major groupings of these parasites such as Distomata, Monostomata, Gasterostomata, and Amphistomata were named for the general body structure of adult trematodes, most notably, the number and arrangement of holdfasts. As life cycles of digenetic trematodes were elucidated, many instances of convergent evolution that resulted in similar adult morphology of unrelated trematodes were discovered. Taxa such as Monostomata and Amphistomata are no longer considered valid, but still are retained today to designate informally convenient assemblages of trematodes without implying any phylogenetic relationship. Old phylogenetic proposals were replaced with schemes based upon similarities of life history patterns among trematodes. Life history studies were also responsible for the awareness of parasitologists that a species concept based solely upon morphological criteria was subject to error. Two separate situations exist in which the morphological relationships of parasitic forms may not reveal the proper determination of biological species. In the first instance, parasites may be morphologically identical but differ from one another with regard to behavior, physiology, geographic distribution,

host specificity, pathogenicity, or other factors. This type of parasitic variation is not related to the present study of the variation within Alloglossidium. For those who wish to pursue this subject, an excellent review of such concepts as strains, races, incipient species, and sibling species as they apply to parasitic organisms can be found in Dogiel (1964). The second aspect of the species problem that reveals the shortcomings of a morphological species concept is host induced intraspecific variation. In such instances, members of a single biological species may show great morphological diversity when parasitizing different host species. These morphological differences may be so great that the description of separate species would be warranted if only morphological criteria were considered.

Beaver (1937) was first to determine experimentally the existence of intraspecific variation of a digenetic trematode. In his classic monograph on Echinostoma revolutum, he demonstrated that the morphology of the adult trematode was affected by the host species, and the age and diet of the host. As a result of his experimental infections of avian and mammalian hosts, he synonomized many species of Echinostoma as host induced variants, or ecotypes of E. revolutum. Rankin (1937) studied variation in natural infections of Brachycoelium in salamanders in North Carolina. He demonstrated that variations between the various specimens of Brachycoelium formed a continuous gradient of characters and he concluded that eighteen described species were synonomous with B. salamandrae.

Other workers have reported similar variation in other trematodes which possess a broad host specificity. Differences in morphology have most often been reported in trematodes such as Fasciola and Dicrocoelium which are of medical or veterinary importance. Noble and Noble (1971)

discussed the cosmopolitan distribution of F. hepatica which may, as an adult, infect sheep, cattle, pigs, rodents, elephants, kangaroos and man. Upon comparing various specimens of F. hepatica, they reported that "specimens of F. hepatica taken from a cow could not be assigned on the basis of morphology alone, to the same species as F. hepatica taken from a guinea pig." Dicrocoelium dendriticum, according to Stunkard (1957), is very dissimilar when taken from its various species of hosts. Haley (1962) reviewed those factors which other workers have found to affect the morphology of trematodes, cestodes, and parasitic nematodes. Among the causes of variation were host species, host age, host diet, number of worms present, previous exposure to the parasite, and presence of another parasite. Watertor (1967) studied the intraspecific variation of adult Telorchis bonnerensis in amphibian and reptilian hosts. T. bonnerensis, a common parasite of amphibians, decreased in size and was delayed in development in reptilian hosts, but successive generations in amphibian hosts were normal in size and rate of development. She reported that the commonly used morphological characters of the genus such as extent of vitellaria, position of the ovary, and extent of the cirrus sac are unreliable in delimiting species within the genus Telorchis.

These and other reports of host induced morphological variation in trematodes were an indication to parasitologists of the errors inherent in a species concept based exclusively on morphology. The integration of genetics into evolutionary concepts represented a potential for change. The possibilities of the use of the new systematics for digenetic trematodes was presented by Stunkard (1957) in his discussion of intraspecific variation in parasitic flatworms.

"According to Mayr, Linsley and Usinger (1953), the old systematics was organized around the central position of the species, typologically conceived, morphologically defined, essentially non-dimensional; whereas in the new systematics the morphological definition is replaced by a biological one, which takes cognizance of ecological, geographical, genetic, and other factors, and populations have become the basal taxonomic units. The species is portrayed as consisting of variable populations, capable of interbreeding. Accordingly, species are defined as groups of actually or potentially interbreeding populations which are reproductively isolated from other such groups."

Stunkard reported Mayr's awareness of the limitations of a single definition of species in this quotation from Genetics, Paleontology and Evolution. "It is rather hopeless to arrive at a satisfactory species definition if one wants to include in a single species concept, apomicts, hybrid flocks, obligatory hermaphrodites and asexual organisms. A fairly stable definition may be arrived at for bisexual animals."

Stunkard further states that Dobzhansky, in Genetics and the Origin of Species expressed a view similar to Mayr's. "It is not surprising that the groups of organisms recognized as being uncommonly 'difficult' from the standpoint of delimiting species have proved to be mainly those in which asexual reproduction, apogamy, or self-fertilization are the only, or the chief, modes of propagation."

As a parasitologist, Stunkard (1957) was acutely aware of the potential for application of the new systematics to endoparasites:

"The 'genetic species' of the new systematics has nothing of practical value for the student of parasitic flatworms. These

worms ordinarily do not occur in interbreeding populations; they are hermaphroditic and self-fertilizing; most of them have alternation of hosts, and often two, three, or even four hosts in the life-cycle, in which the sexual generation may be followed by a series of asexually produced generations in very different hosts. For more than forty years I have been occupied with the study of these worms and I have yet to find any formula to replace the one given by Looss (1902) to define species, genera, and higher taxonomic units. The situation is one of the most exasperating and challenging in systematic zoology. The worms cannot be maintained apart from their hosts, and the influence of the host on the parasite can never be completely assessed. At one time it was believed that each species of parasitic flatworm is limited to a single host-species, but the development of the same species in hosts as distant as reptiles, birds, and mammals has disposed of that idea. Indeed, in most instances we do not know the extent of possible hosts. When an investigator is confronted with similar worms from different hosts, he has no precise way of determining whether he is dealing with one or more than one species. In such variable worms, which have no skeletal structure, and in which the shape is modified by the contraction of different sets of muscles; which may become sexually mature at one-fourth the maximum size and continue to grow as long as they live; in which the morphology is dependent of the degree of maturity; and in which the location and shape of organs are influenced by extension and contraction of the body, by accumulation of genital products (spermatozoa in the seminal vesicle

> or eggs in the uterus), or even by accumulation of fluid in the excretory vesicle; specific determination of a particular individual may pose an almost insoluble problem."

Although the opinion given by Stunkard expresses succinctly the dilemma of the parasitologists, Yamaguti (1971) offers a more positive approach to a useful taxonomic concept through life history and ecological studies of digenetic trematodes.

> "Generally speaking, the external and internal anatomy of adult trematodes is subject to modification as a result of their adaptation to their respective habitats. Under these circumstances, the adult morphology of the Digenea, which provides an important clue to generic differentiation, is overweighed by the life cycle patterns, but because the life histories of large numbers of groups are entirely unknown or only partly known, it is impossible to classify all Digenea on the basis of biotic relationships alone. Therefore, we must resort to the only alternative left to us, i.e., a new scheme of classification based on combinations of adult morphology and ecology, with some speculations on those groups whose life history is unknown."

I agree with Stunkard's argument that because of the hermaphroditism and potential for self fertilization of digenetic trematodes, that a species concept based upon the criteria of reproductive isolation is beset with problems. I believe that a modern species concept for digenetic trematodes should integrate evidence obtained from the study of any aspect of the biology of these organisms. The utilization of life history and ecological studies, as advocated by Yamaguti, has the

greatest potential for trematode taxonomy and systematics, but evidence from physiological, biochemical, karyological, ethological, and other studies are also of great importance.

I have attempted in my study, to apply ecological data to determine whether the various morphological forms of Alloglossidium are distinct biological species or whether each morphological forms belongs to the same biological species and differs structurally as a consequence of the unique environmental conditions provided by a particular host species. I have concluded from evidence derived from experimental infections and differences in the distribution of the morphological species of Alloglossidium that each is also a separate biological species. Each biological species of Alloglossidium, with the exception of A. corti, has a very restricted specificity with regard to the second invertebrate host employed in the life cycle. These various morphological forms of Alloglossidium cannot, therefore, be host induced variants because of the lack of broad host specificity. Even in the exceptional instances when normal host specificity breaks down and one species of Alloglossidium parasitizes an abnormal host, the species of Alloglossidium shows no structural modifications in its abnormal host. Eichler (1966) has proposed the term allohospitalic that "pertains to two or more related parasitic species that do not occur on the same host species, but live on different (though, in most cases, closely related) host species, even though these host species may live in quite the same localities." The significance of this term is that, according to Eichler "the new terms 'synhospitalic' and 'allohospitalic' refer to the fact that - as far as we know - microevolution (or speciation) in biting lice [and in other parasitic taxa] is more influenced by host (or 'hospitalic')

isolation than by geographic distribution."

Progenesis in Digenetic Trematodes

Progenesis was first applied in 1924 to digenetic trematodes by Dollfus (in Stunkard, 1959), to describe the phenomenon of precocious genital activity. The term paedogenesis of Von Baer, referring to precocious sexual maturity of the reproductive organs while an organism is still in the condition of a larva or even an embryo (De Beer, 1958), is closely related to progenesis. I will however, discuss only progenesis since, as Baer has stated (in Babero, 1972) "it is difficult to define. . . a 'paedogenetic metacercaria' since the latter, when producing eggs even when encysted are no longer larvae".

Different degrees of progenesis have been reported. Early genital development of metacercariae is found in many families of digenetic trematodes. In some instances, metacercariae become sexually mature while encysted in the intermediate host and are capable of producing eggs in which develop viable miracidia. Several trematodes have been described which can complete their life cycle without the involvement of a vertebrate host. Parasitologists in general, have referred to these latter trematodes as being progenetic. I believe however, that this term cannot justifiably be applied until a thorough study has been conducted concerning the probable origin of all forms which become gravid in an invertebrate host. Two possible explanations exist with regard to the origin of progenetic trematodes. In the first instance, trematodes that become gravid in invertebrates may represent the primitive life cycle pattern of a particular group, in which case, these organisms cannot correctly be termed progenetic. In the second instance, if trematodes that become sexually mature in invertebrates can

be shown to have originated from a stock that underwent precocious genital development, then such forms may be called progenetic trematodes. More properly, those trematodes that have eliminated, through this process, the requirement of a vertebrate host, may be termed descendants of progenetic trematodes, since at their state of evolution, development in the invertebrate can now be considered normal, and no longer precocious.

Two opinions exist among parasitologists with regard to the phenomenon of progenesis in relation to the phylogeny of digenetic trematodes. Stunkard (1957) expressed one opinion in this statement: "Progenesis occurs in members of many families; its distribution and incidence are too extensive to be explained as a mutation; rather it appears to be a relict, the persistance of an earlier state, which obtained before sexual maturity was so largely deferred to definitive vertebrate hosts."

The strongest evidence for regarding progenesis as an advanced condition of digenetic trematodes is presented by Cable (1965) and Pearson (1972). Cable cites the work of La Rue (1957) who proposed a phylogenetic scheme of digenetic trematodes which has received wide acceptance by parasitologists. La Rue divided the digenetic trematodes into two superorders on the basis of the presence or absence of an epitheliated excretory bladder, and stated that Anepitheliocystidia was the more primitive of the two. Cable (1965) has shown that the great majority of progenetic trematodes belong to the more advanced superorder Epitheliocystidia. He stated that groups with an epitheliated excretory bladder typically have a metacercaria which encysts in a second intermediate host, with a large amount of development typically being required before the metacercaria is morphologically and physiologically capable of infecting the definitive host. "It [metacercaria] may grow and dif-

ferentiate to the extent that organs of the adult are well formed; even progenetic development of the metacercaria to full sexual maturity with the production of many eggs is being reported from more and more families in which the bladder is epitheliated."

Pearson (1972) has devised theoretical patterns of the evolution of life cycles among digenetic trematodes. His schemes are in agreement with the arguments of Cable with regard to the interposition of an encysted metacercarial stage in a second intermediate host, between a precocious cercarial stage and a sexually mature adult in a definitive host. In his scheme, the advantage of relegating a greater portion of development to the metacercaria rather than to the cercaria was that it allowed the potential for quantitatively greater cercarial production by the first intermediate host. Ultimately, the increase in the amount of development which took place in the metacercaria led to precocious gonadal development and the attainment of sexual reproduction. Pearson recounts the effect that progenesis has upon the life cycle pattern of trematodes, and its phylogenetic implications. "The attainment of sexual maturity in the second intermediate host has led in some cases to the elision of the definitive host and the reduction of the life-cycle from three hosts to two." "According to the phylogeny advanced herein, a metacercaria approaching sexual maturity is advanced, whereas one that changes little if any from the cercarial body is primitive. Except in the case where retrogression of metacercarial development is indicated, it follows that if in two groups a series from 'primitive' to 'advanced' metacercaria can be discerned, then the two have presumably been separated for a long time and can be related phylogenetically only at an early stage. It also follows that a group with a 'primitive'

metacercaria cannot be derived from a group with an 'advanced' metacercaria. Such considerations may help, in conjunction with other features, to illuminate the interrelationships of digenetic groups."

While a few digenetic trematodes that attain maturity in a host that would normally be considered an intermediate host in the life cycle, such as the sexually mature cercaria of Proterometra, Cable and Pearson have argued convincingly that the majority of progenetic trematodes are advanced. I have employed their rationalization to construct a phylogeny of the genus Alloglossidium. The phylogenetic relationships if this genus have been established on the basis of the morphology of the adults, on host specificity, and upon the relative degree of progenesis which each species exhibits (Figure 13).

I have stated previously that the genus Alloglossidium can be divided into two groups on the basis of adult morphology. These groups also indicate the types of hosts parasitized by the species of Alloglossidium: A. macrobdellensis and A. hirudicola in leeches and A. corti, A. renale, and A. progeneticum in arthropods. The life cycle of A. corti is typical of the pattern of that of the Plagiorchioidea and Macroderoididae and thus represents the most primitive life cycle of Alloglossidium. The cercaria of A. corti has slight genital development. Encystment of the cercaria in a wide range of second intermediate hosts is followed by precocious genital development, but this does not include the attainment of sexual maturity. Occasional encystment of A. corti metacercariae in the daughter sporocyst reported by McMullen (1938) is another example of precocious development of A. corti. After the ingestion of the metacercarial cyst of A. corti by a vertebrate definitive host, sexual maturity occurs.

The life cycle of A. progeneticum represents an advanced condition in relation to that of A. corti. In A. progeneticum, the cercaria penetrates a crayfish host, an elimination of the broad host specificity of A. corti. Encystment of A. progeneticum occurs in a typical macroderoidid fashion, but the precocious development of the metacercaria culminates with the attainment of sexual reproduction and the production of enormous eggs which remain confined within the cyst wall. Ingestion of the gravid metacercariae results in excystment and the establishment of adult A. progeneticum infections within the intestine of a vertebrate definitive host. The life cycle of A. renale represents the ultimate condition of this group of Alloglossidium which consists of A. corti, A. progeneticum, and A. renale. Cercarial penetration of A. renale is restricted to a single species of host, the fresh-water shrimp, Palaemonetes kadiakensis. Development begins immediately without encystment. Eggs containing viable miracidia are liberated from the shrimp through the excretory pore of the host. The vertebrate host has been completely eliminated from the life cycle of A. renale.

The life cycle pattern of Alloglossidium from leeches represents the second divergence from the typical macroderoidid cycle which is exemplified by A. corti. The life cycle of A. hirudicola is unknown, but adults occur in the ceca of the leech Haemopis sp. A. hirudicola has not been reported from vertebrates. The life cycle of A. macrobdellensis has been reported by Corkum and Beckerdite (1975). Cercarial penetration is restricted to Macrobdella ditetra. A. macrobdellensis undergoes the remainder of its life cycle within the leech as unencysted coelomic worms, followed by encystment in the crop and some organogenesis. After a period of development in the crop cysts, A. macrobdellensis excysts and

migrates to the intestine. In the intestine, A. macrobdellensis becomes sexually mature and eggs containing viable miracidia are liberated through the anus of the leech host. A. macrobdellensis does not require a vertebrate host in its life cycle.

Effect of Progenesis on Host Specificity

The specificity of a digenetic trematode can be expressed in various ways at different stages of development and with respect to the various hosts in the life cycle. Dogeil (1964) has expressed the normal state of host specificity in parasitic animals:

> "the parasites are most narrowly specific to their hosts at the stage when they undergo vigorous development of the reproductive organs, or when they reproduce sexually or in the parthenogenetic manner, i.e., when they undergo extensive morphological and physiological changes. In contrast, at those stages which do not develop within the host, or do not reproduce sexually, but only grow and show some degree of organogenesis, they are characterized by wider specificity. Many parasites, therefore, are widely specific towards their second intermediate hosts, and usually their carrier hosts. This situation is fairly definitely demonstrated by the trematodes and some cestodes. For example, the parthenogenetic stages of the trematodes develop, as a rule, only in one or two species of molluscs. The cercariae, on the other hand, having left the mollusc, attack many hosts. The metacercariae, which develop only slightly, show the widest specificity. . . The host range of the adult trematodes is again narrower than that of their

> metacercariae. In those instances (e.g. Microphalliae), when the metacercariae undergo considerable development in the host (development of all their reproductive organs and sometimes even the formation of eggs), their specificity to the second intermediate host is very narrow. The adult microphallids live in many vertebrate species, passing through hardly any development in their intestine, only producing eggs and rapidly leaving the host."

The same phenomenon of reversal of normal host specificity is precisely what occurs in the progenetic members of Alloglossidium. A. corti metacercariae do not mature in the second intermediate hosts. These metacercariae have the typical broad specificity of macroderoidid metacercariae and can develop in mayflies, damselflies, dragonflies, and crayfishes. In each of the species of Alloglossidium which reaches sexual maturity in an invertebrate host, the host specificity has become narrowly restricted. A. macrobdellensis and A. hirudicola can develop in only two genera of leeches. A. progeneticum has been reported from only one species of crayfish and A. renale can infect only the freshwater shrimp, Palaemonetes kadiakensis. Therefore, each species of Alloglossidium exhibits a restricted specificity for the host in which sexual maturity is attained.

Figure 13 presents a proposed phylogenetic scheme for Alloglossidium based upon morphology, host specificity and degree of progenesis.

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TABLE I. Differential Morphological Characters of Adult *Macroderoides* and *Alloglossidium*

Macroderoides	*Alloglossidium*
1. Body elongate	1. Body elongate, fusiform or ovoid
2. Tegument heavily spined	2. Tegument lightly or moderately spined
3. Cirrus long and thick	3. Cirrus weakly developed
4. Cirrus sac very large, greatly overreaching acetabulum posteriorly	4. Cirrus sac very small to moderate, usually only slightly overreaching acetabulum posteriorly
5. Ovary widely separated from acetabulum	5. Ovary only slightly posterior to acetabulum except in very elongated species
6. Vitellaria confined to hindbody	6. Vitellaria extend anteriorly to cecal bifurcation
7. Excretory bladder short, extending forward to posterior testis	7. Excretory bladder long, extending forward to ovary

TABLE II. Differential Morphological Characters of *Macroderoides* and *Alloglossidium* Cercariae

Macroderoides	*Alloglossidium*
1. Stylet unshouldered	1. Stylet with distinct shoulders on lateral and ventral surfaces
2. Refractile droplets present in parenchyma	2. Refractile droplets absent
3. Penetration glands prominent without vital staining	3. Penetration glands conspicuous only with vital staining
4. Ceca rudimentary, preacetabular	4. Ceca complete, extending to caudal pocket
5. Tail finfold present or absent	5. Tail finfold absent
6. Caudal pocket small and ovoid	6. Caudal pocket large, with broad lateral expansions and robust spines
7. Lateral excretory ducts usually obscure	7. Lateral excretory ducts usually conspicuous
8. Reproductive system undifferentiated	8. Reproductive system partially differentiated

TABLE III. Differential Morphologic Characters based on a Paratypic Series of *Alloglossidium renale* and a Topotypic Series of *A. progeneticum*

A. renale	*A. progeneticum*
1. Body robust, 1,880 long by 790 wide	1. Body fusiform, 1,590 long by 465 wide
2. Oral sucker 150 long by 160 wide	2. Oral sucker 100 long by 100 wide
3. Acetabulum 135 long by 135 wide	3. Acetabulum 100 long by 100 wide
4. Prepharynx short and undilated	4. Prepharynx long and dilated posteriorly
5. Ceca thin walled, extremely dilated	5. Ceca thick walled, undilated
6. Anterior testis 115 long by 120 wide	6. Anterior testis 90 long by 95 wide
7. Posterior testis 140 long by 135 wide	7. Posterior testis 100 long by 110 wide
8. Cirrus sac 160 long by 55 wide, seldom overlaps posterior limits of acetabulum	8. Cirrus sac 175 long by 50 wide, typically overlaps acetabulum posteriorly
9. Ovary 265 long by 260 wide	9. Ovary 155 long by 145 wide
10. Vitellaria extensively overlapping ceca ventrally and dorsally, but absent from median longitudinal third of body	10. Vitellaria overlapping ceca ventrally but rarely dorsally, more confined to lateral margins of body

TABLE III continued.

	A. renale	A. progeneticum
11.	Uterus - a. Coils completely filling body behind posterior testis b. ascending arm of uterus with several coils, only slightly inflated with eggs c. more than two uterine loops passing between testes	Uterus - a. a single descending and ascending loop behind testis b. ascending arm of uterus sigmoid, extremely inflated with eggs c. only two uterine loops passing between testes
12.	Eggs 28 long by 15 wide	Eggs 25 long by 14 wide
13.	Never encysted in antennary gland	Always encysted in antennary gland

TABLE IV. Summary of the Collections of the Species of Alloglossidium at Selected Localities.

Host	Head of Island	St. James	Brusly	Vacherie
Ictalurus spp.	A. corti	A. corti	A. corti	(1)
Noturus spp.	(1)	Absent	A. corti	(2)
Procambarus spp.	A. corti	Negative	A. corti	A. renale
P. (Pennides) spp.	Absent	Absent	Absent	Absent
Cambarellus spp.	Negative	Negative	Negative	Negative
Orconectes spp.	Absent	Absent	A. corti	Absent
Macrobdella ditetra	A. macrob-dellensis	Absent	A. macrob-dellensis	A. macrob-dellensis
Palaemonetes kadiakensis	A. renale	A. renale	Negative	A. renale
Helisoma trivolvis	A. macrob-dellensis	Negative	A. macrob-dellensis	A. macrob-dellensis

	Pierre Pass	Sorrento	Bogalusa
Ictalurus spp.	A. corti	A. corti	(2)
Noturus spp.	(2)	(2)	(1)
Procambarus spp.	(1)	(1)	A. corti (4)
P. (Pennides) spp.	Absent	Absent	A. corti (4)

TABLE IV continued.

Host	Pierre Pass	Sorrento	Bogalusa
Cambarellus spp.	(1)	(1)	Absent
Orconectes spp.	(2)	(2)	A. corti (4)
Macrobdella ditetra	A. macrob-dellensis	A. macrob-dellensis	(2)
Palaemonetes kadiakensis	A. renale	A. renale	A. renale A. corti (5)
Helisoma trivolvis	(1)	A. macrob-dellensis	(1)

	Baton Rouge	Watkinsville, Ga.
Ictalurus spp.	Negative	A. corti (6) A. progeneticum (6)
Noturus spp.	Absent	(2)
Procambarus spp.	Negative	Absent
P. (Pennides) spp.	Absent	A. progeneticum
Cambarellus spp.	Absent	Absent
Orconectes spp.	Absent	Absent
Macrobdella ditetra	Absent	Absent
Palaemonetes kadiakensis	Negative	Absent

TABLE IV continued.

Host	Baton Rouge	Watkinsville, Ga.
Helisoma trivolvis	Negative	(2)

(1) Host present, but insufficient data to determine presence or absence of *Alloglossidium*

(2) Insufficient collections made to determine presence or absence of host

(3) One specimen of *Procambarus acutus acutus* contained in six *A. renale*

(4) Very heavy incidence and prevalence of *A. corti* metacercariae

(5) Two *Palaemonetes kadiakensis* had one *A. corti* metacercariae each, encysted in antennary gland

(6) Concurrent infection of adult *A. corti* and *A. progeneticum* in intestine of *I. nebulosus*

TABLE V. Key to the Species of *Alloglossidium*

1a. Testes greater than one half body width 2

1b. Testes less than one half body width 4

2a. Intestinal ceca terminating near level of posterior testis; parasites of leeches 3

2b. Intestinal ceca extending well past posterior testis; adult in intestine of catfishes . . . . . *A. corti*

3a. Prepharynx long; cecal bifurcation midway between oral and ventral suckers . . . . . *A. macrobdellensis*

3b. Prepharynx short; cecal bifurcation near acetabulum . . . . . *A. hirudicola*

4a. Ascending arm of uterus highly inflated and sigmoid in shape; adults encysted in antennary gland of crayfish or free in intestine of catfishes . . . . . *A. progeneticum*

4b. Ascending arm of uterus only slightly inflated and with several small coils; adults free in antennary gland of freshwater shrimp . . . . . *A. renale*

Figure 1. *Alloglossidium corti*, whole mount

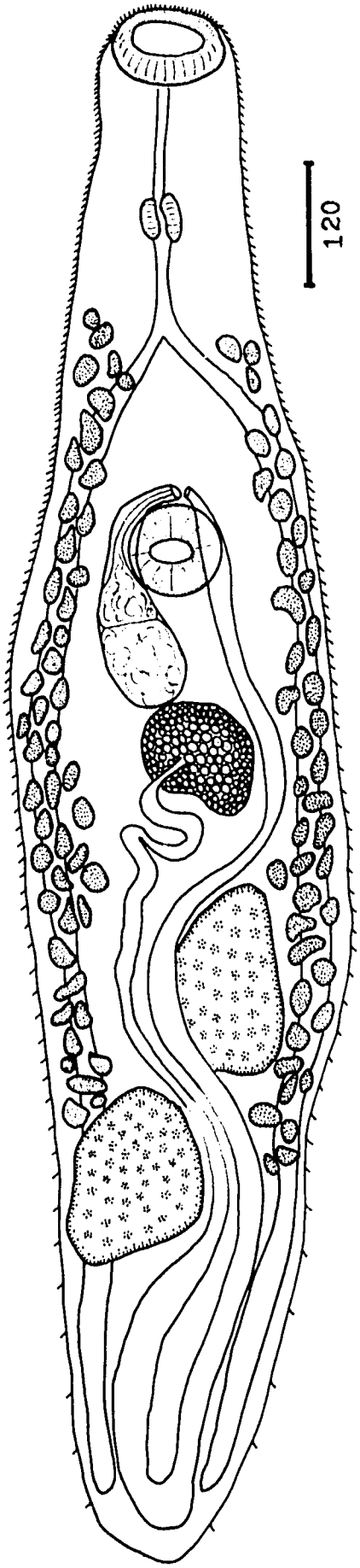

120

Figure 2. *Alloglossidium macrobdelliensis*, whole mount

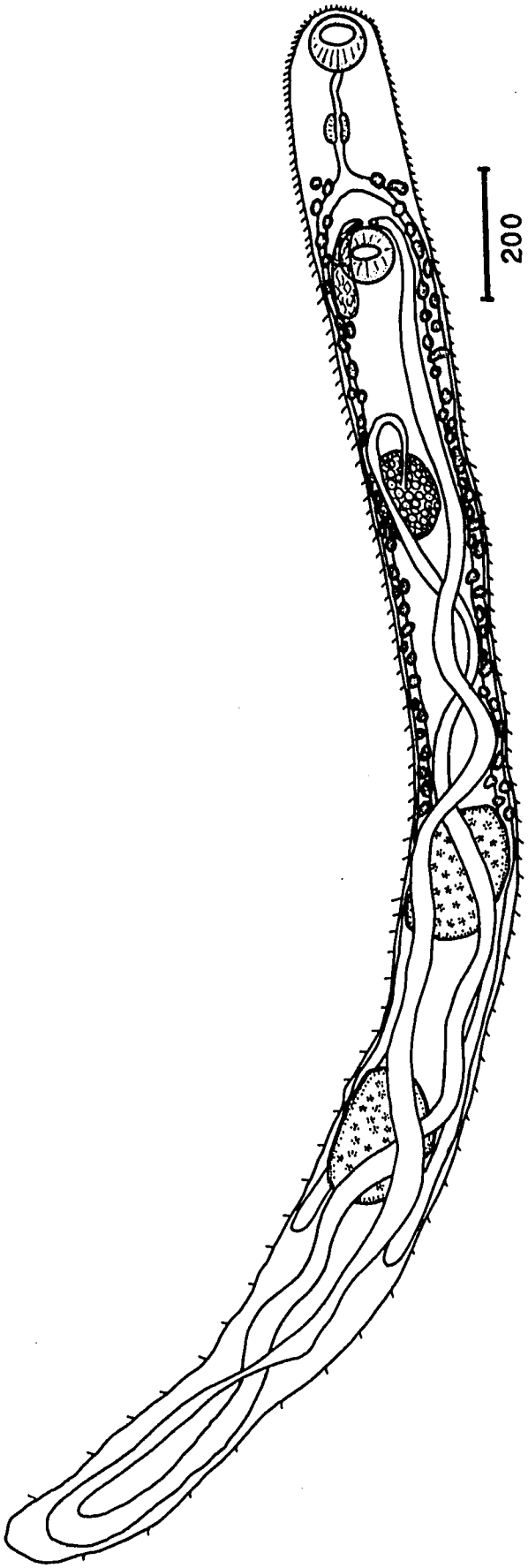

200

Figure 3. Alloglossidium progeneticum, whole mount

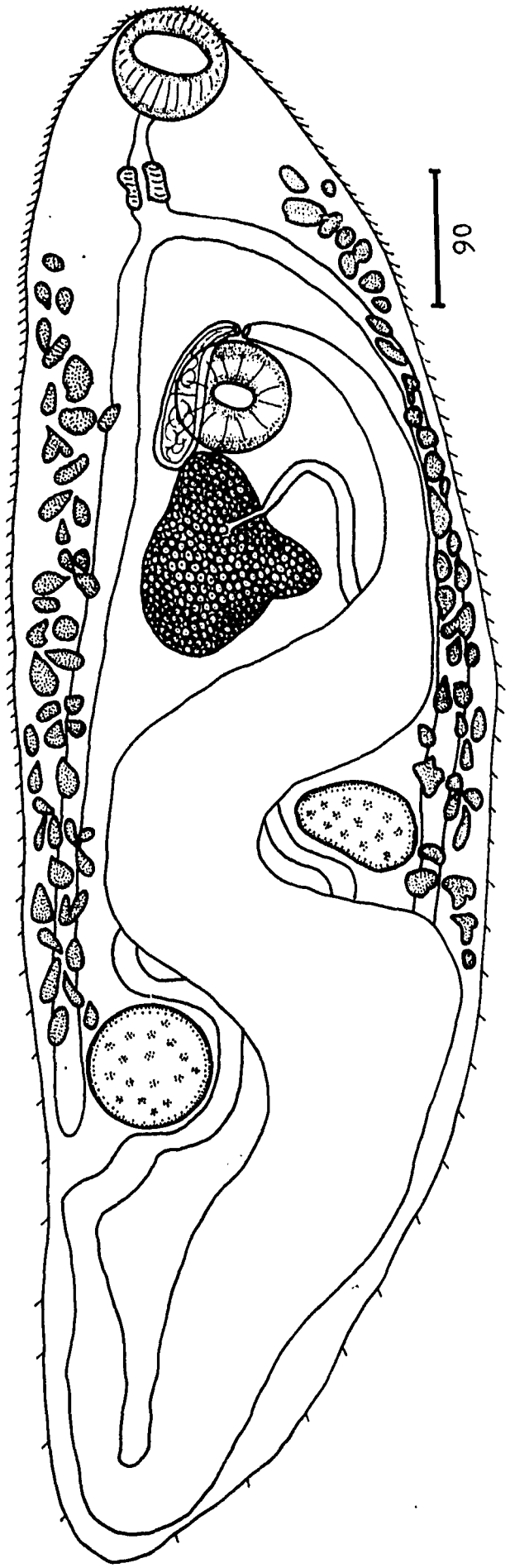
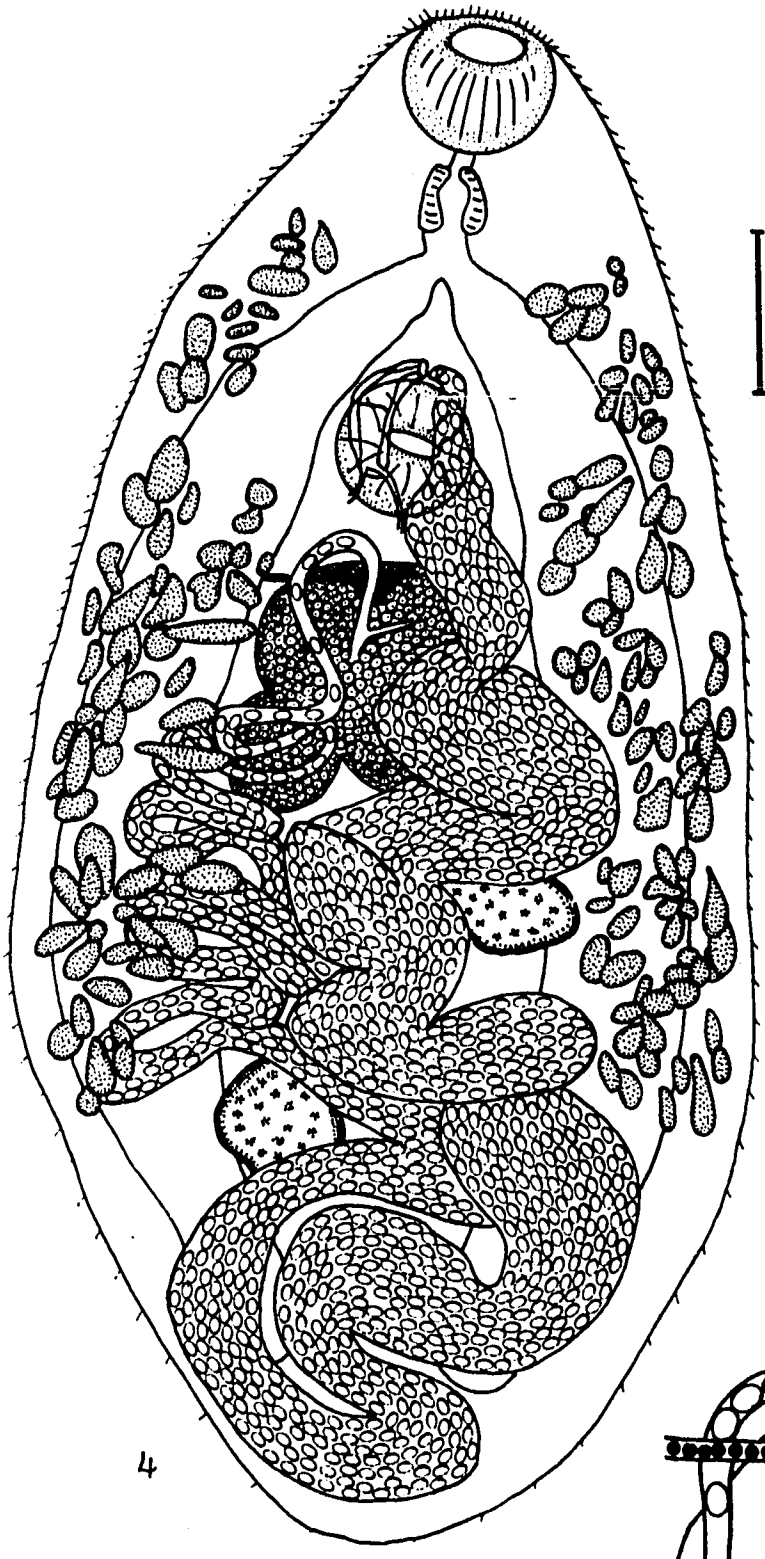

90

Figure 4. Alloglossidium renale, whole mount

Figure 5. Alloglossidium renale, genitalia



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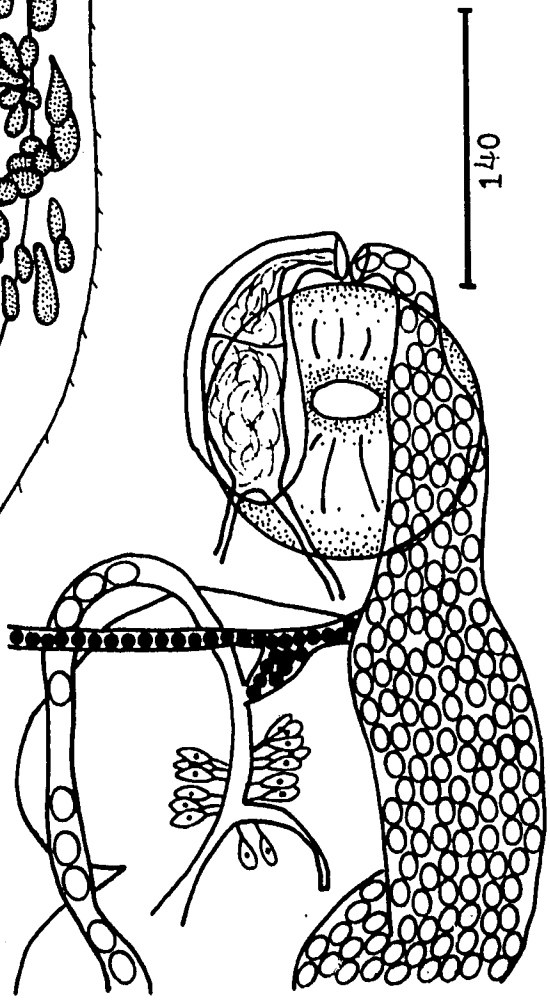


5

Figure 6. Hypothetical example illustrating a method of determining ecological independence.

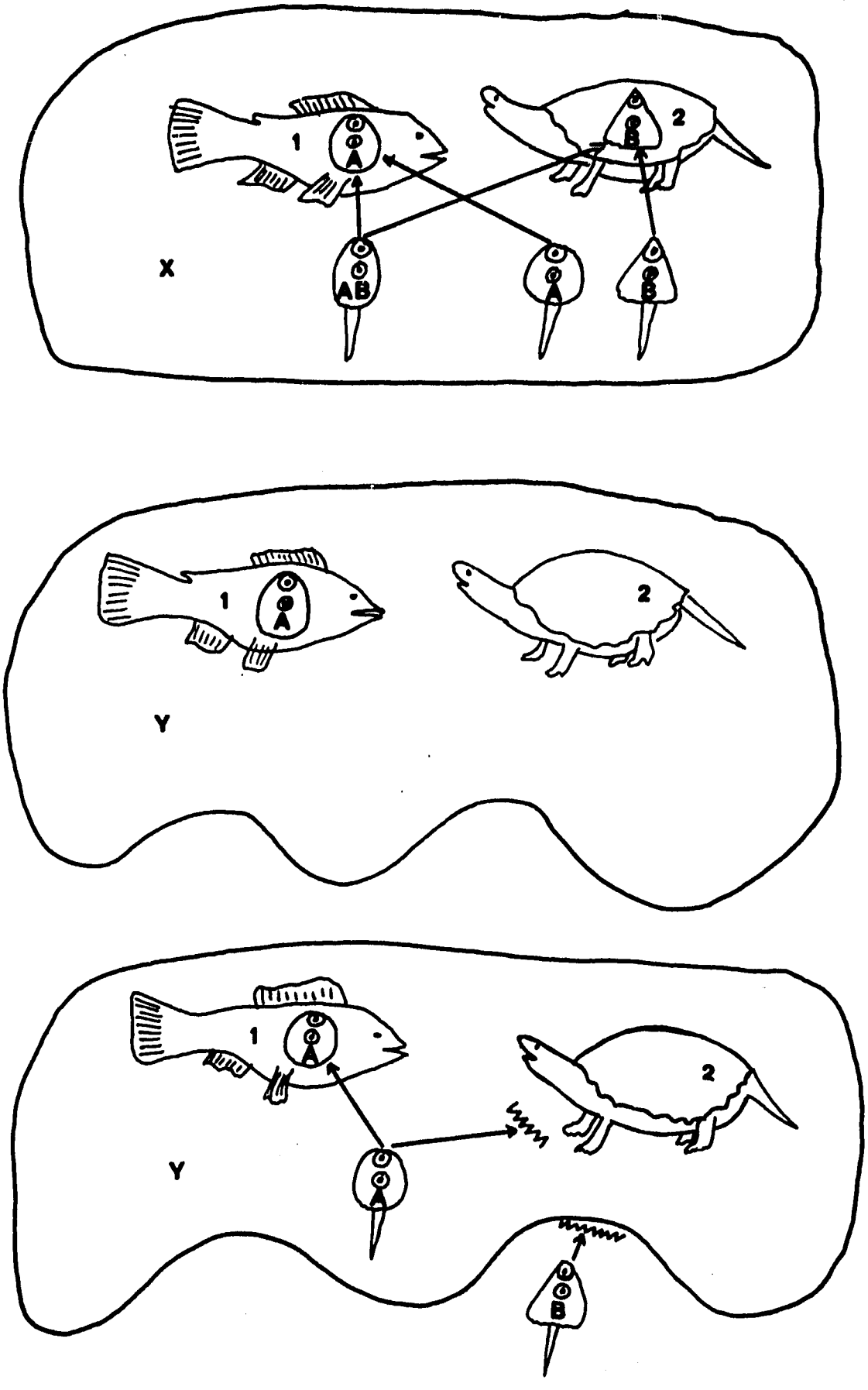

1
A
2
B
X
AB
A
B
1
A
2
Y
1
A
2
Y
A
B

Figure 7. Seasonal incidence of *Alloglossidium renale* in *Palaemonetes kadiakensis* at St. James, Louisiana

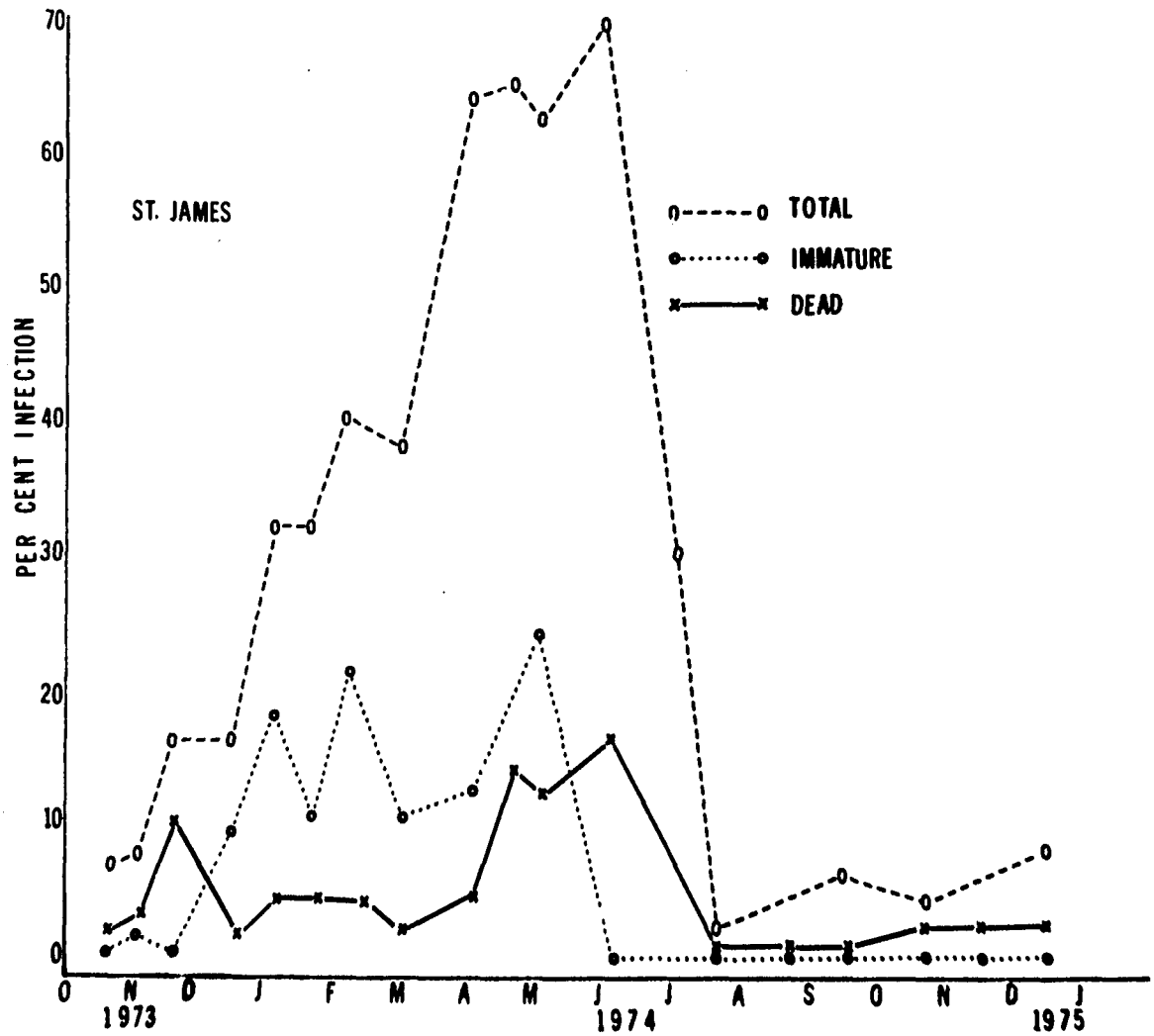

ST. JAMES
TOTAL
IMMATURE
DEAD
PER CENT INFECTION
70
60
50
40
30
20
10
0
O N D J F M A M J J A S O N D J
1973
1974
1975

Figure 8. Seasonal incidence of *Alloglossidium renale* in *Palaemonetes kadiakensis* at Head of Island, Louisiana

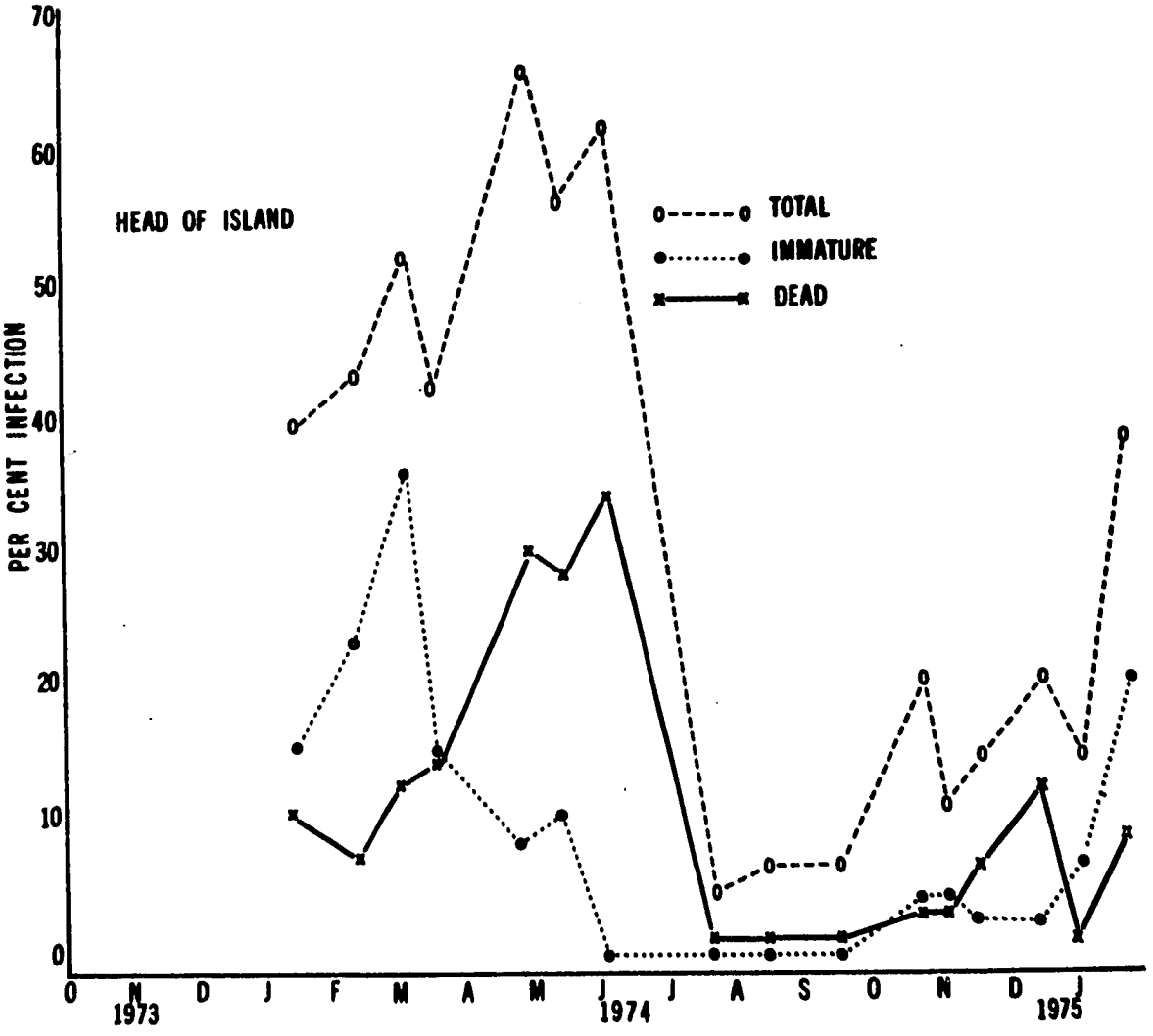

HEAD OF ISLAND
TOTAL
IMMATURE
DEAD
PER CENT INFECTION
70
60
50
40
30
20
10
0
O N D J F M A M J J A S O N D J
1973
1974
1975

Figure 9. Uninfected antennary gland of Palaemonetes kadiakensis

Figure 10. Antennary gland of Palaemonetes kadiakensis infected with Alloglossidium renale

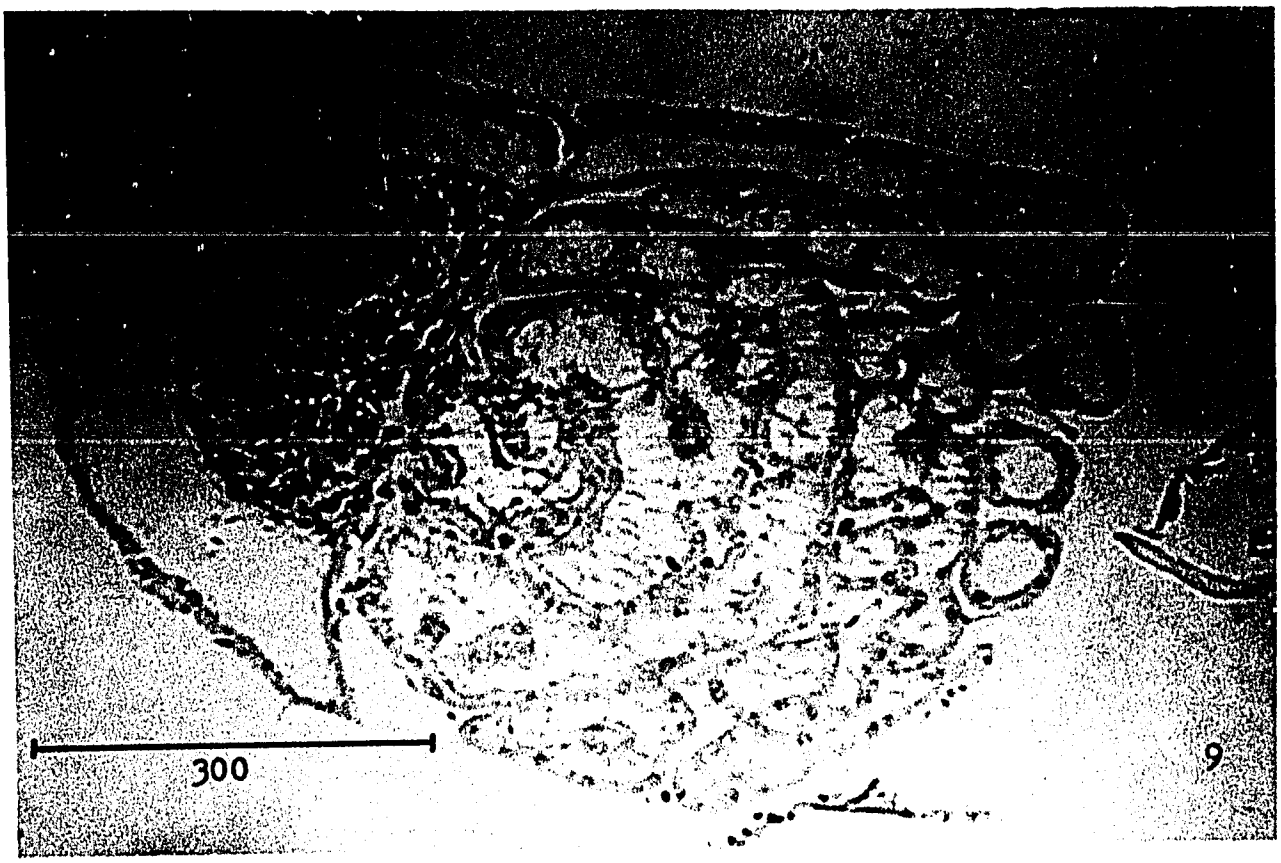

300
9

Figure 11. Erosion of the tissue of the antennary gland of *Palaemonetes kadiakensis* by the tegumental spines of *Alloglossidium renale*

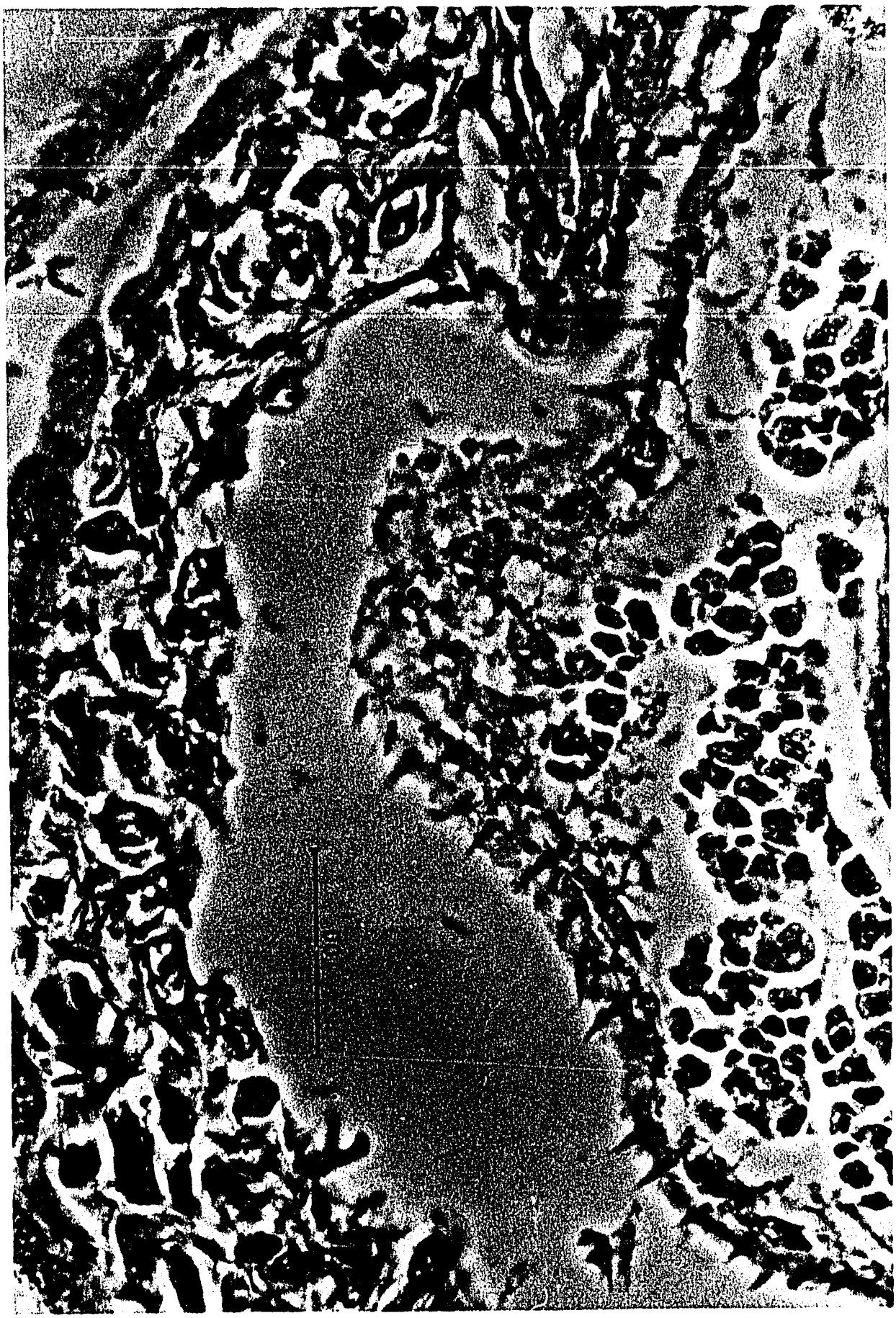


Figure 12. Ecological Relationships of the Species of Alloglossidium

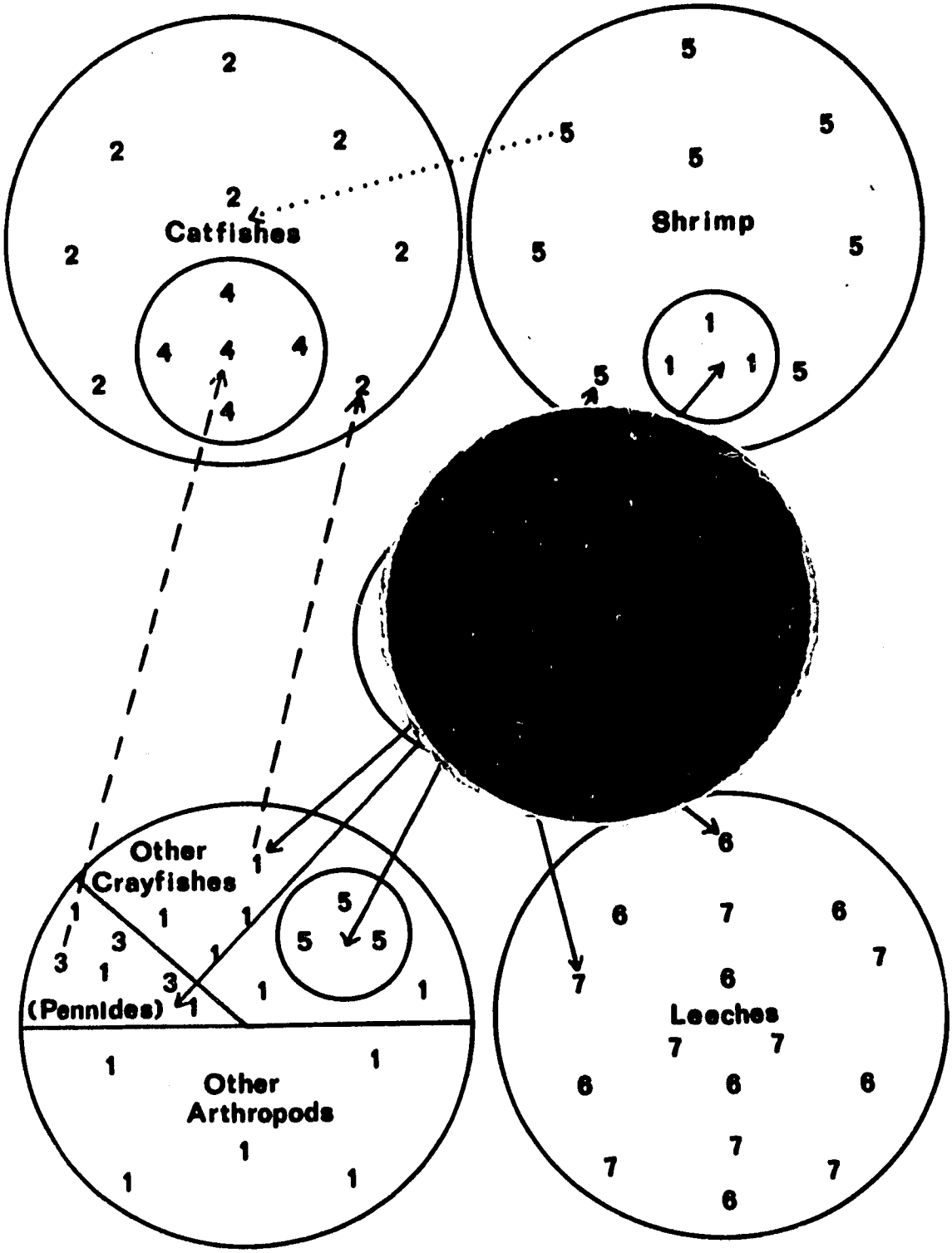
1. A. corti; encysted, immature
2. A. corti; not encysted, mature
3. A. progeneticum; encysted, mature
4. A. progeneticum; not encysted, mature
5. A. renale; not encysted, mature
6. A. macrobdellensis
7. A. hirudicola

Ecological interaction:

— — — — → predation; parasite transferred

..... → predation; parasite not transferred

————→ cercarial penetration


Catfishes
Shrimp
Other Crayfishes
(Pennides)
Other Arthropods
Leeches

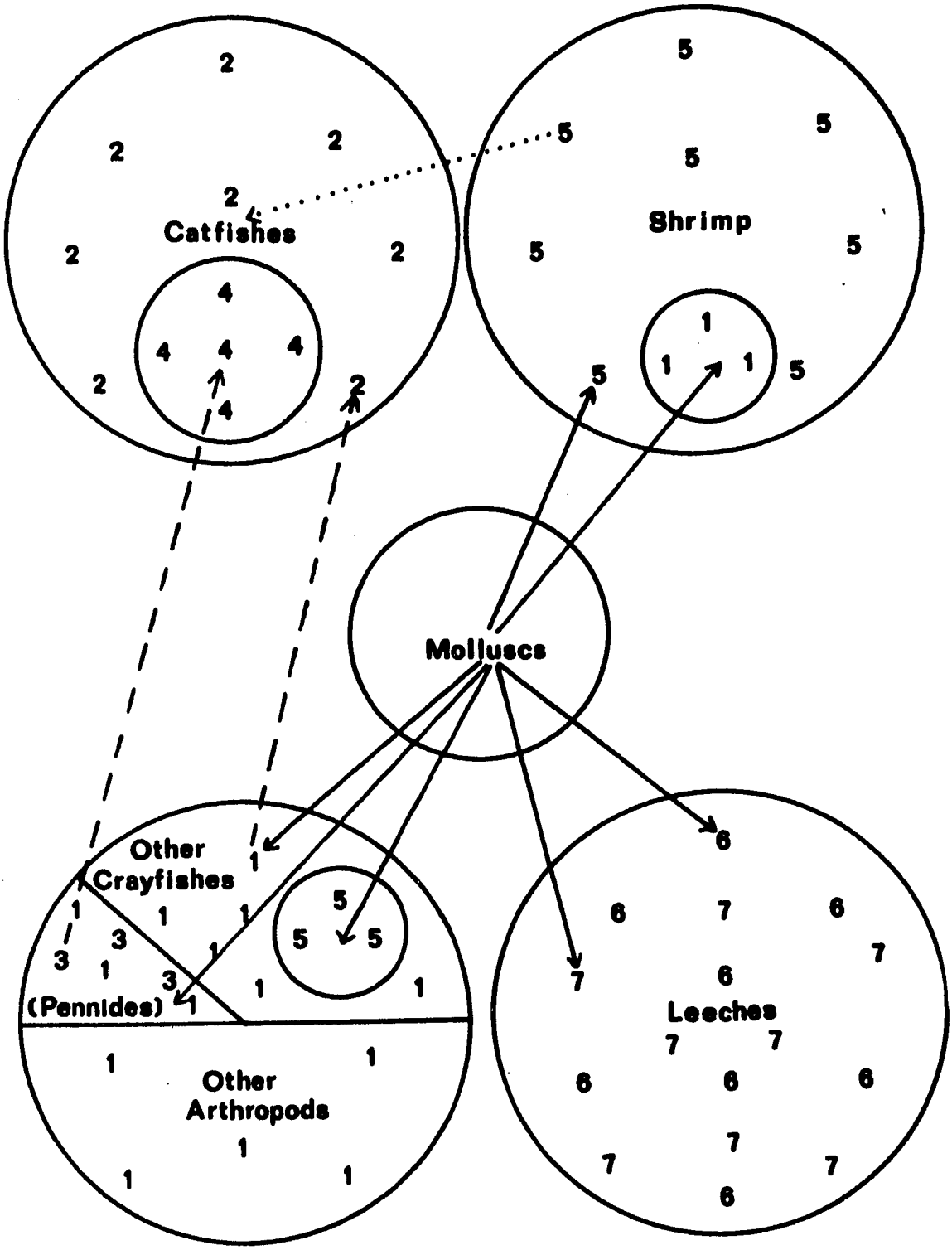
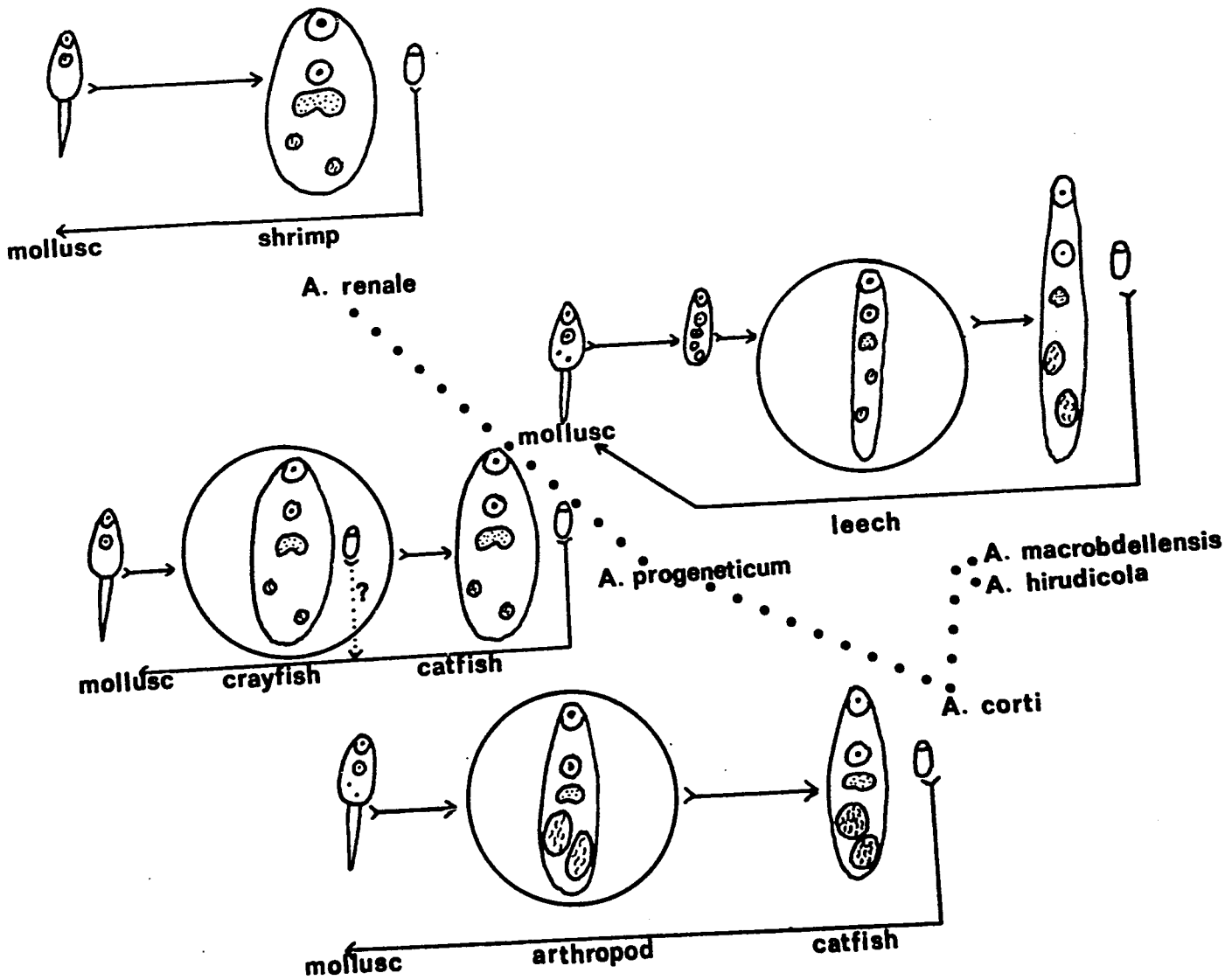

Catfishes
Shrimp
Molluscs
Other Crayfishes
(Pennides)
Other Arthropods
Leeches

Figure 13. A proposed phylogenetic scheme for the species of *Alloglossidium*


mollusc
shrimp
A. renale
mollusc
leech
A. progeneticum
A. macrobdellensis
A. hirudicola
mollusc
crayfish
catfish
?
A. corti
mollusc
arthropod
catfish

VITA

William F. Font, Jr. was born on August 11, 1944. He graduated from Hahnville High School, Hahnville, Louisiana, and received the Bachelor of Science degree from Tulane University in 1966. He has been a student in the Department of Zoology and Physiology since 1970, and received the Master of Science degree in 1972. He is presently a candidate for the Doctor of Philosophy degree.

EXAMINATION AND THESIS REPORT

Candidate: William F. Font, Jr.

Major Field: Zoology

Title of Thesis: An Ecological Approach to the Taxonomy of the Genus Alloglossidium (Trematoda: Macroderoididae)

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

April 4, 1975